

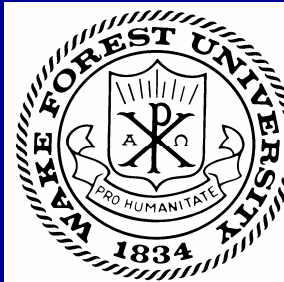
# **Tissue Engineering and Regenerative Medicine**

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**Wake Forest Institute for Regenerative Medicine**

**Wake Forest University School of Medicine**

**Winston-Salem, NC**



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**YOU ARE HERE**



## **Combat Trauma**

**Blunt, penetrating and blast injuries may lead to soft and solid tissue and organ damage**

**Trauma, and its subsequent infection and inflammation all lead to tissue loss**

**Challenge: Replacement Tissues and Organs**

1954, First organ transplant, Boston

Today, Increasing problem: tissue and organ shortage and rejection



**Tissue Engineering**

**Biosurgery**



**Regenerative Medicine**



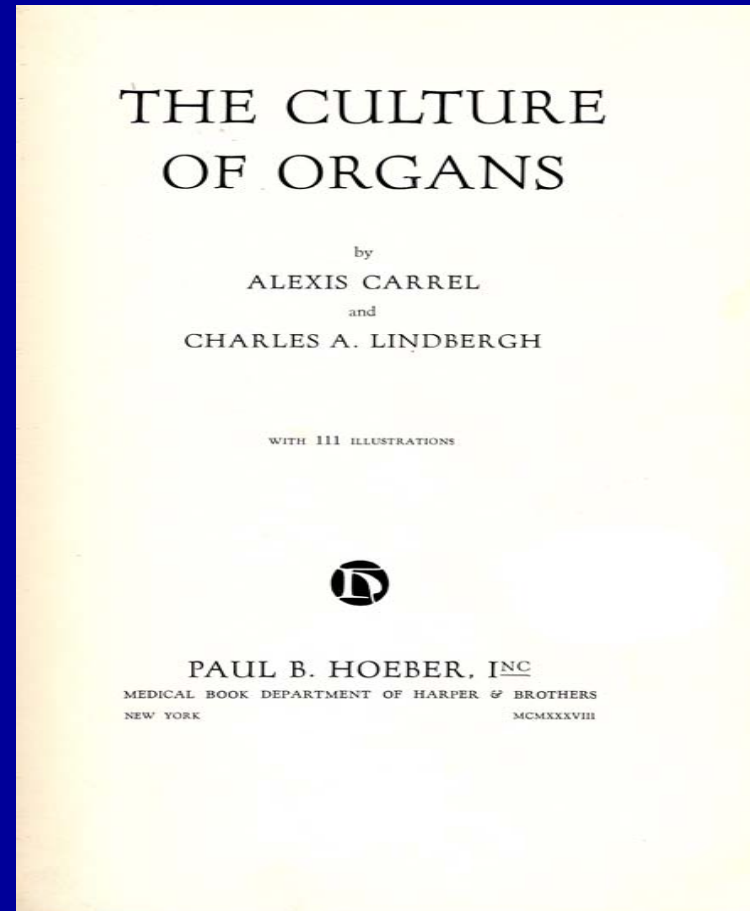
**Cell Therapies**

**Artificial and  
Biohybrid Organs**



# Regenerative Medicine / Tissue Engineering

**Based on the field of cell transplantation (started in 1930s)**



**First clinical application: engineered skin for burn patients, 1981**



# **Regenerative Medicine / Tissue Engineering**

**A field of research for over 60 years. Why so few clinical advances?**

**Inability to expand cells in vitro**

**Inadequate biomaterials**

**Inadequate vascularity**

# **Wake Forest Institute for Regenerative Medicine**

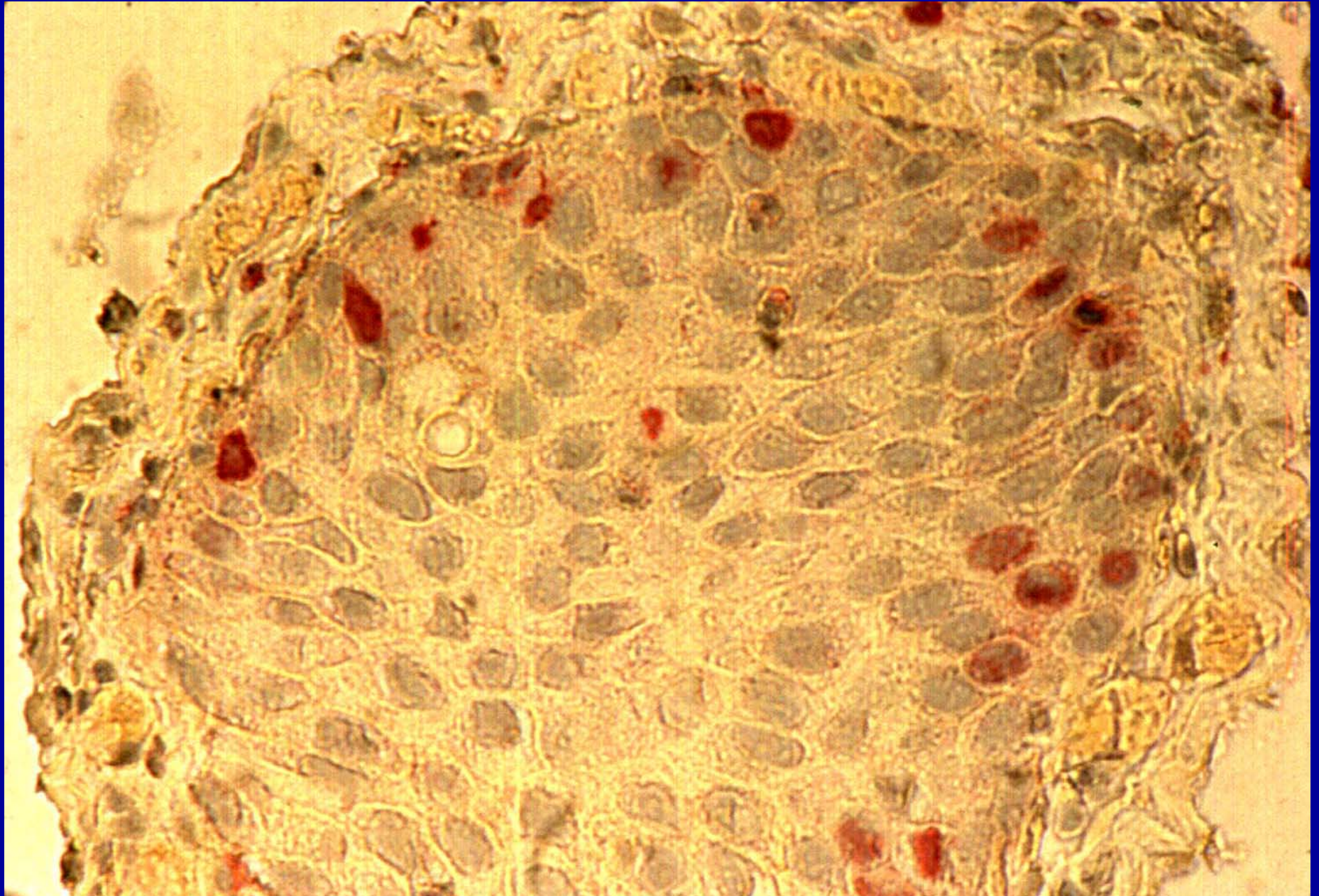
**Growth factor biology**

**Cell Differentiation**

**Molecular mechanisms**

**Cell-matrix interactions**

## Targeted Committed Progenitor Cells



# **Progenitor Cells and Specific Growth Factors:** **Expansion Potential**

**1 cm<sup>2</sup>**

**Day 1 (5 X 10<sup>4</sup> cells)**



**Day 60 (50 X 10<sup>9</sup> cells)**

**Enough cells to cover a football field**

# **Cell Types Grown at the Wake Forest Institute for Regenerative Medicine**

**Heart**

**Kidney**

**Esophagus**

**Bladder**

**Sm/Sk Muscle**

**Cartilage**

**Urethra**

**Vessels**

**Salivary glands**

**Trachea**

**Bone**

**Breast**

**Lung**

**Retina**

**Uterus**

**Nerve**

**Liver**

**Pancreas**



# **CELL DELIVERY VEHICLES**

**Biocompatibility**  
**Cell attachment**  
**Cell viability**

**Degradation curves**  
**Inflammatory responses**  
**Biomechanical properties**

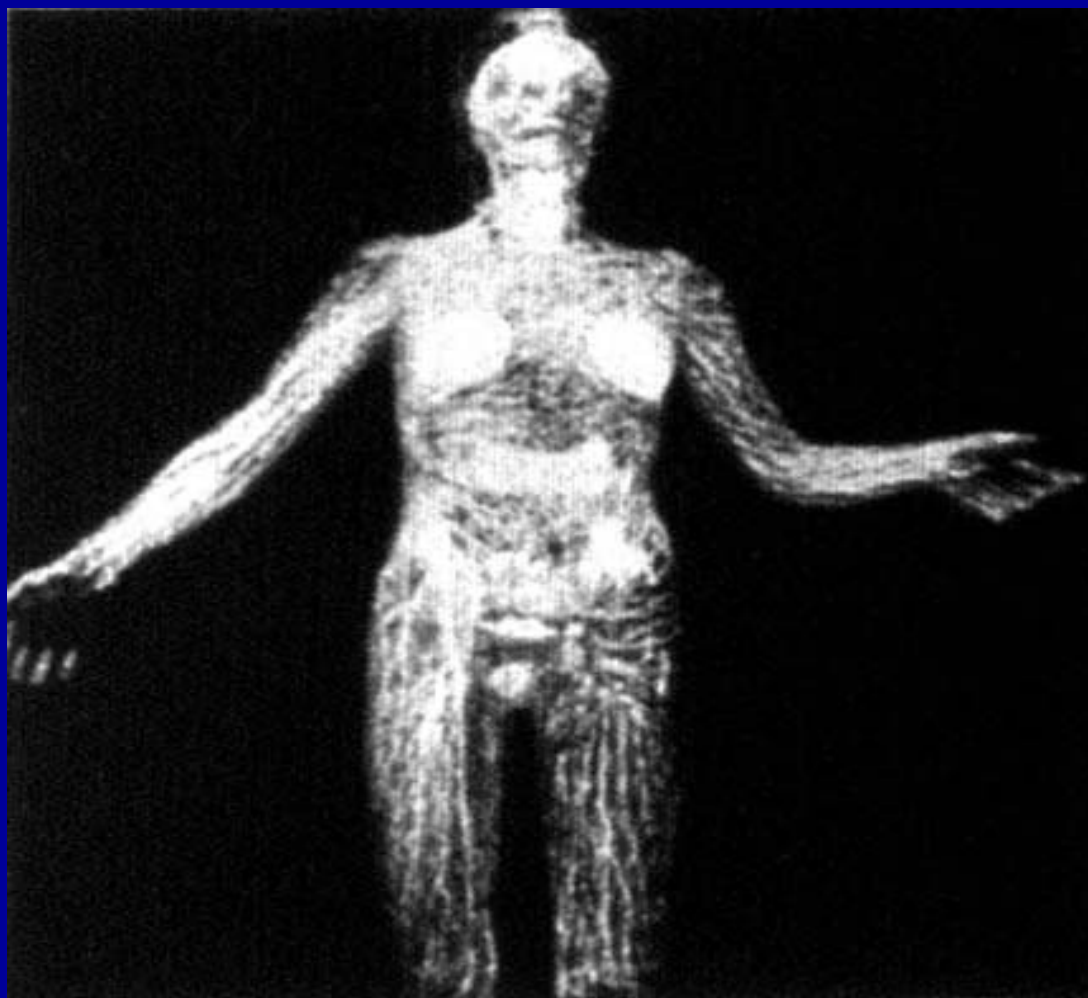
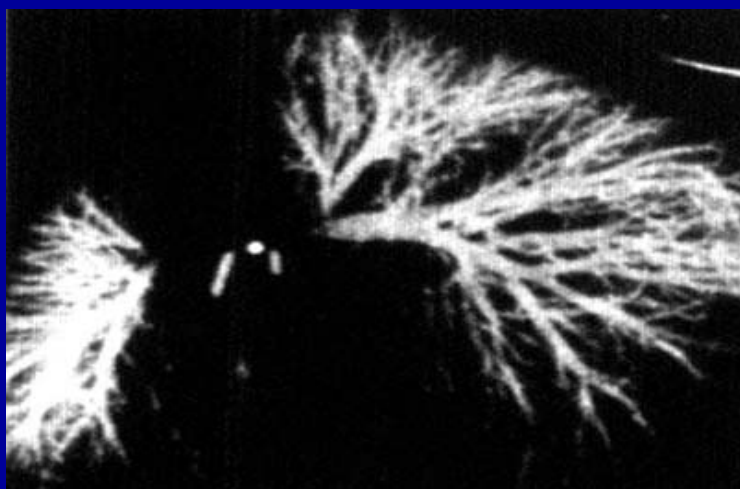
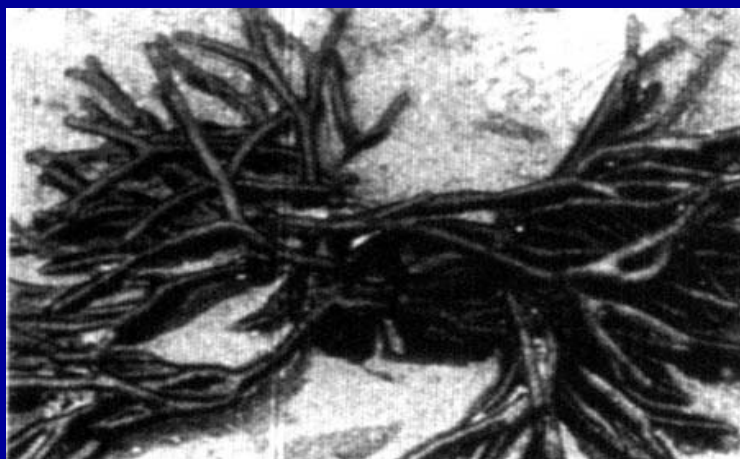
**The scaffold should replicate the biomechanical and structural properties of the tissue being replaced**

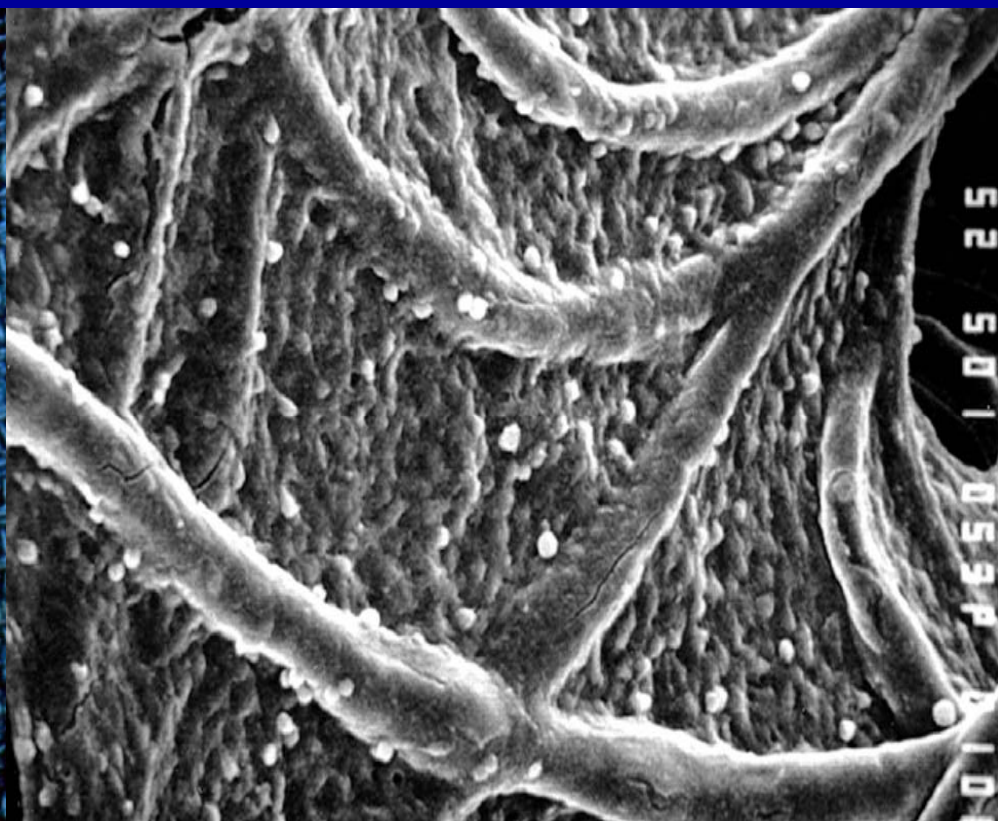
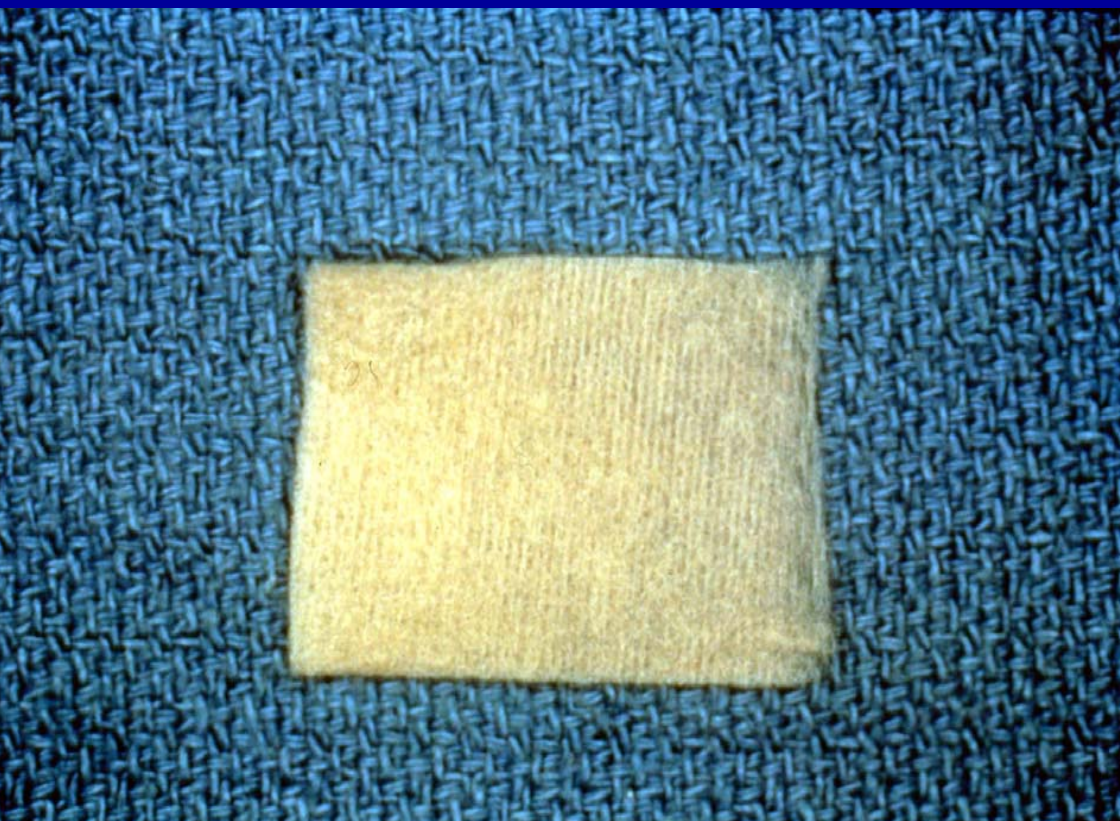
# Vascularity: Problem

**Cells cannot be implanted in volumes greater than 3 mm<sup>3</sup> (the size of a pencil eraser)**

**Nutrition to the cells is limited (limited vascularity)**

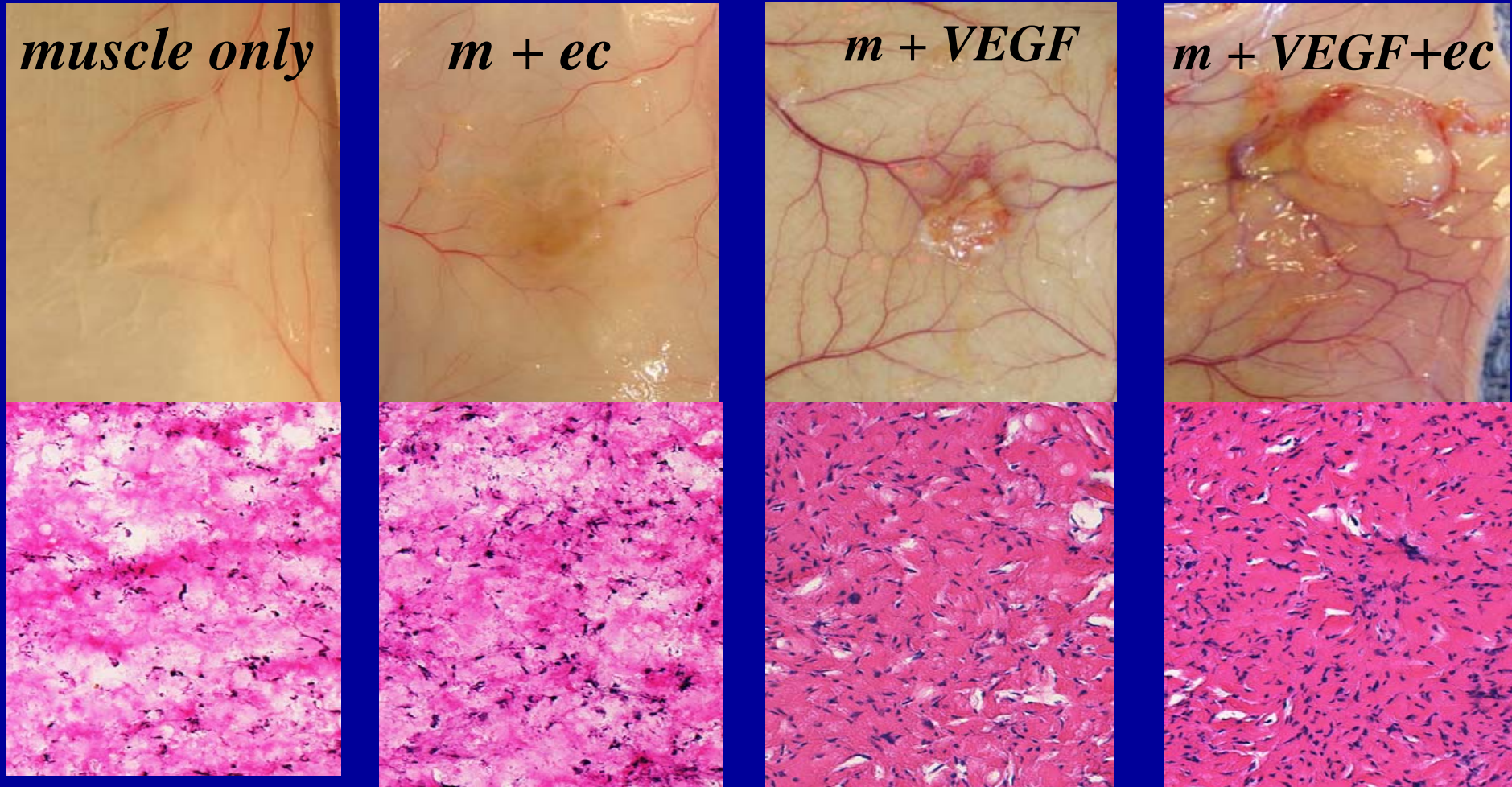








## Tissue Formation in Vivo



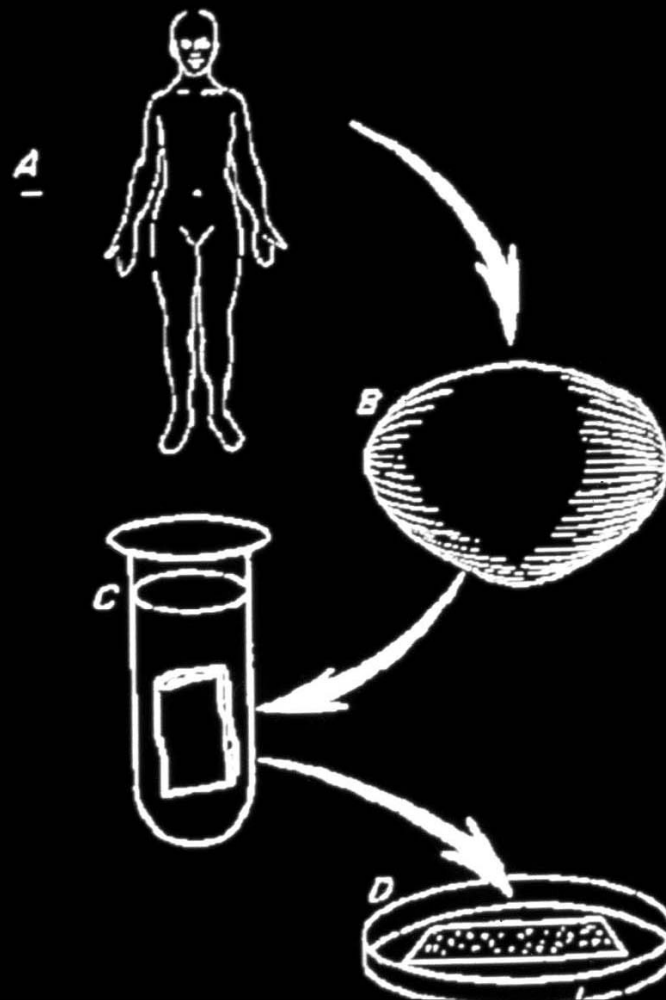
*G Schuch et al, Blood 100:4622, 02.*

*WT Godbey et al, Gene Ther, 03.*

*G Schuch et al, Angiogenesis 5:181, 02.*

*RC Smith et al, Hum Gene Ther 13:697, 02*

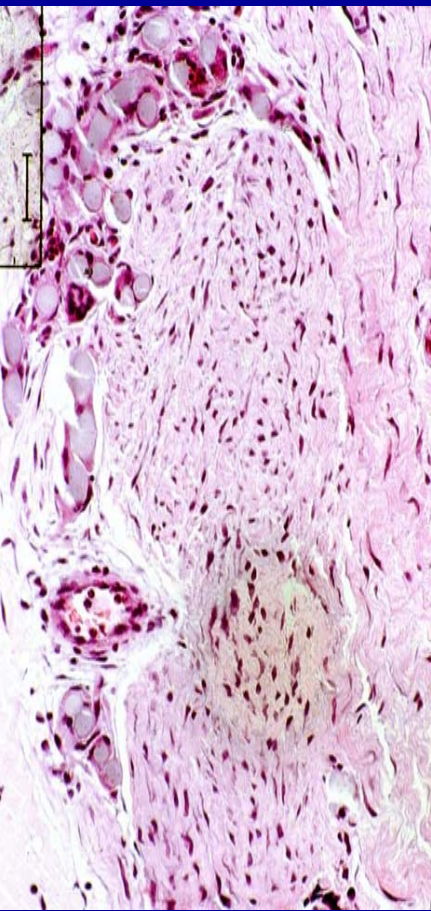
*M Nomi et al, Mol Aspects Med 23:463, 02.*



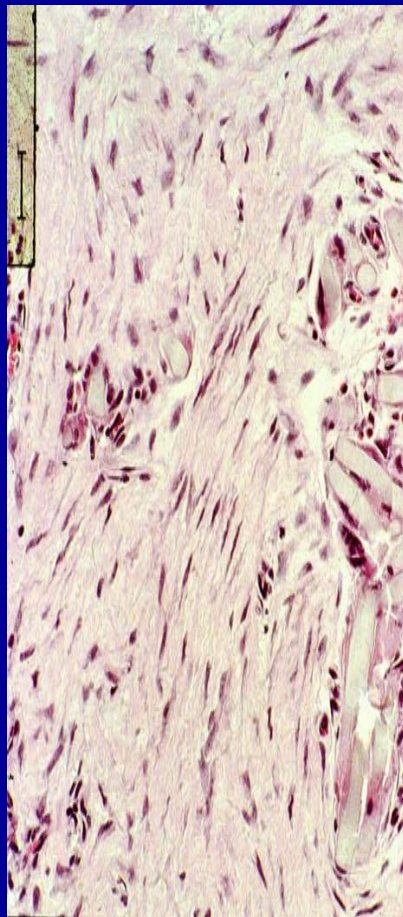


# Building Blocks for the Engineering of Tissues and organs

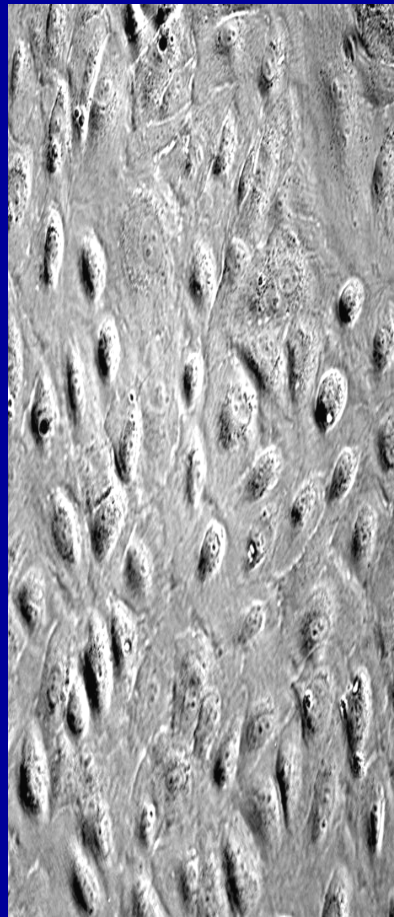
**Muscle**



**Heart mm**



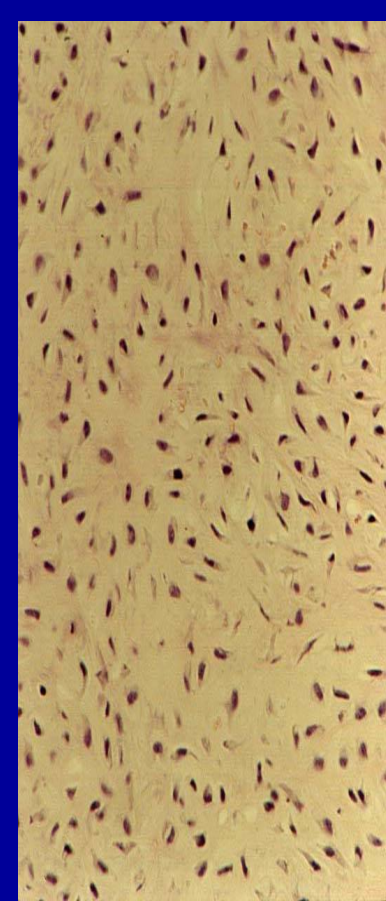
**Endothelium**

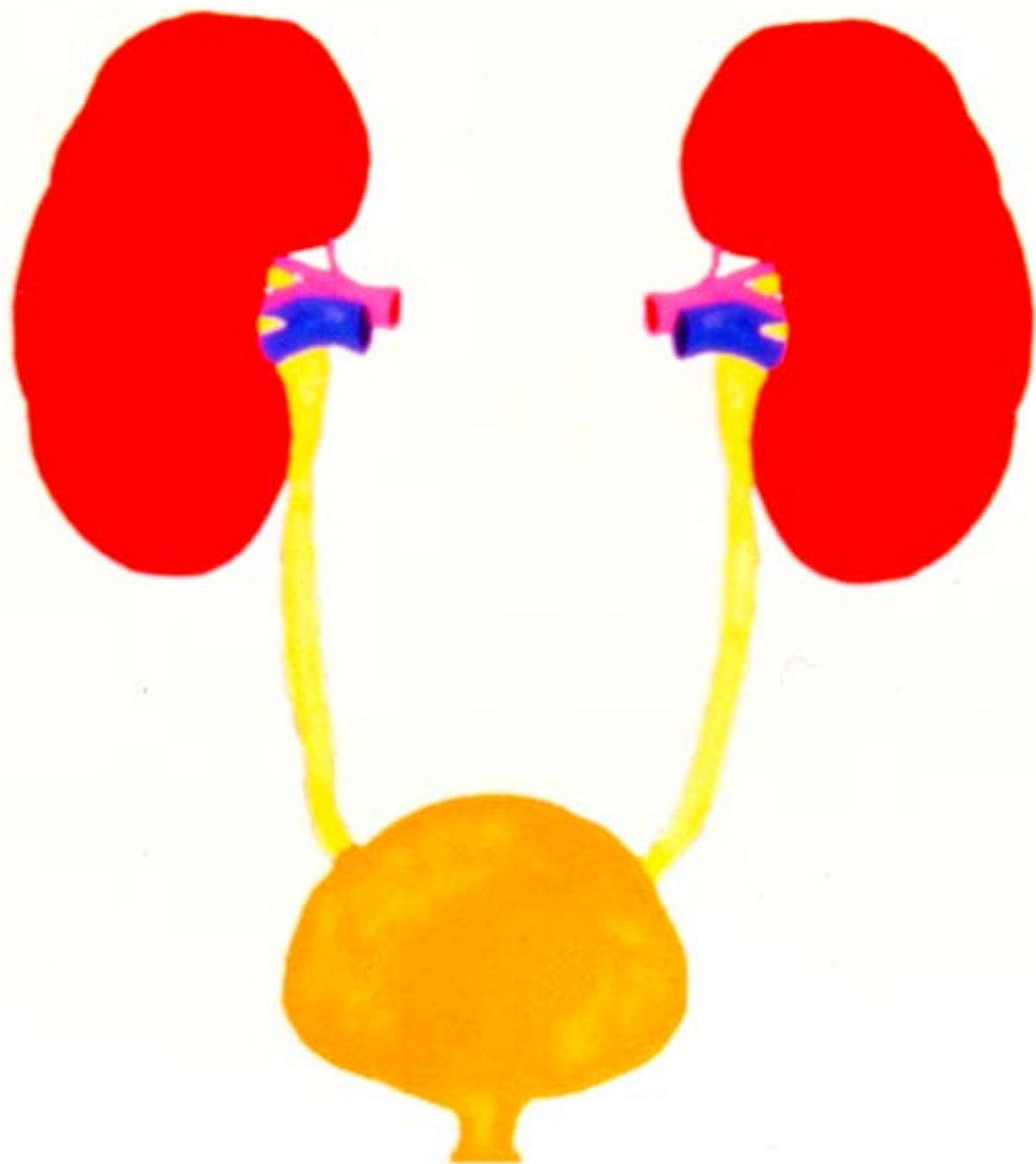


**Bone**



**Cartilage**

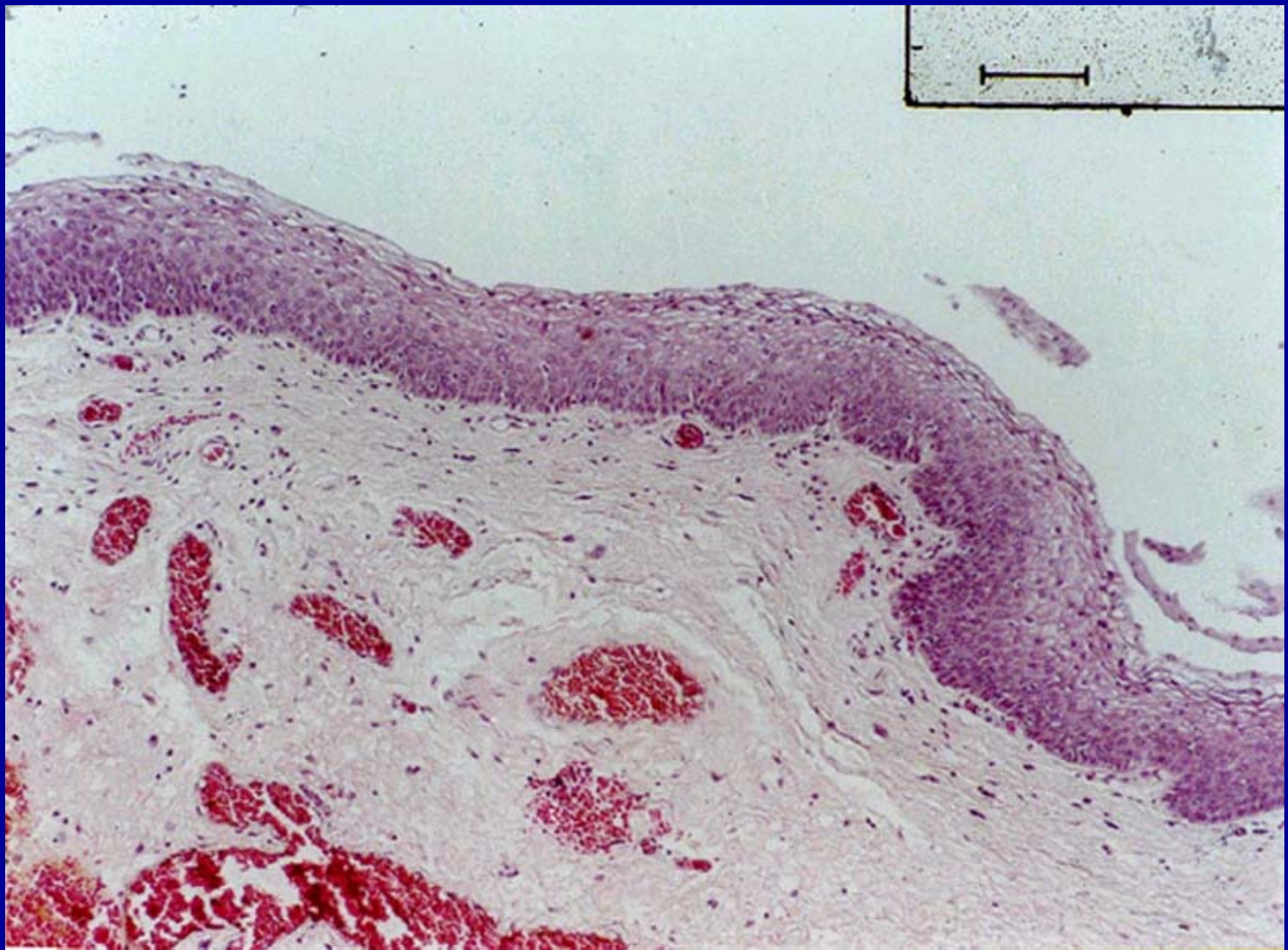














*J Urol 162:1148, 99.*

*F Chen et al, Urology 54:1, 99.*

*RE DeFilippo et al, J Urol 168:1789, 02.*

*AW El-Kassaby et al, J Urol 169:170, 03.*

## **Urethra: Clinical Experience**

**Over 100 patients treated to date**

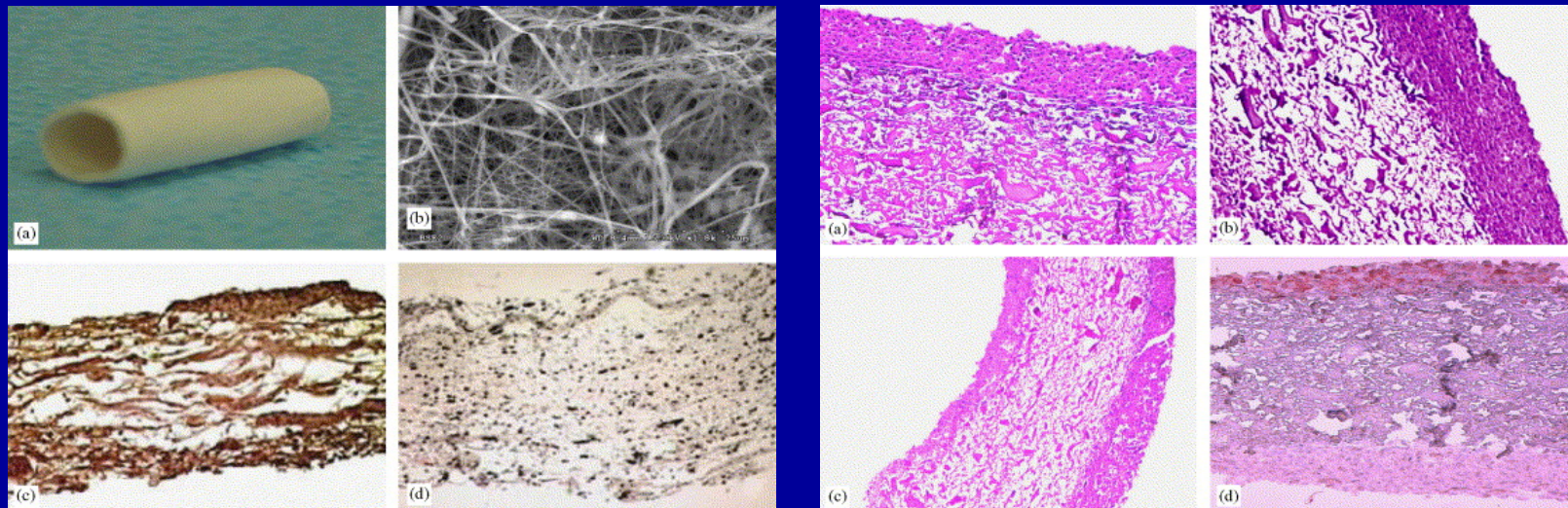
**Over a 5 year follow-up**

**80% Success rate**



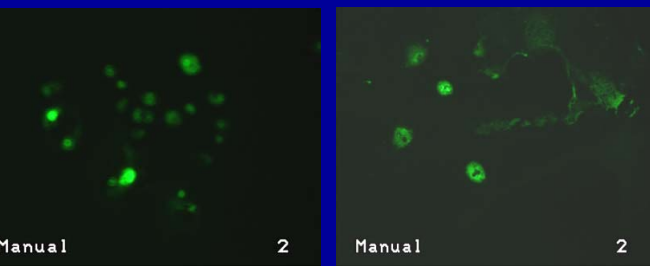
# Fabrication of a vascular substitute

Electrospun nanofiber substrate, with endothelial and smooth muscle cells

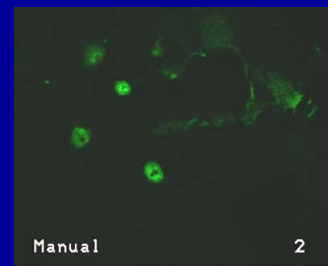


Stitzel et al., *Biomaterials*, 2005.

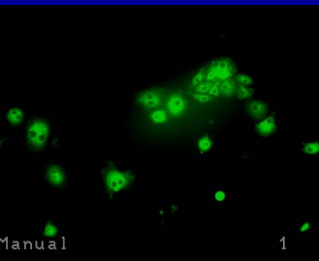
# Peripheral Blood-derived Endothelial Cells for the Creation of Tissue Engineered Blood Vessels



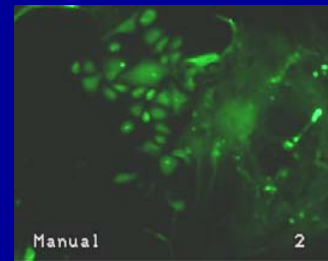
FLK1



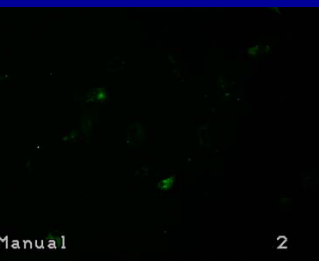
CD31



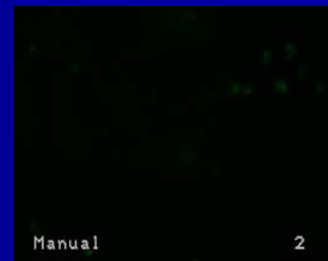
BS1



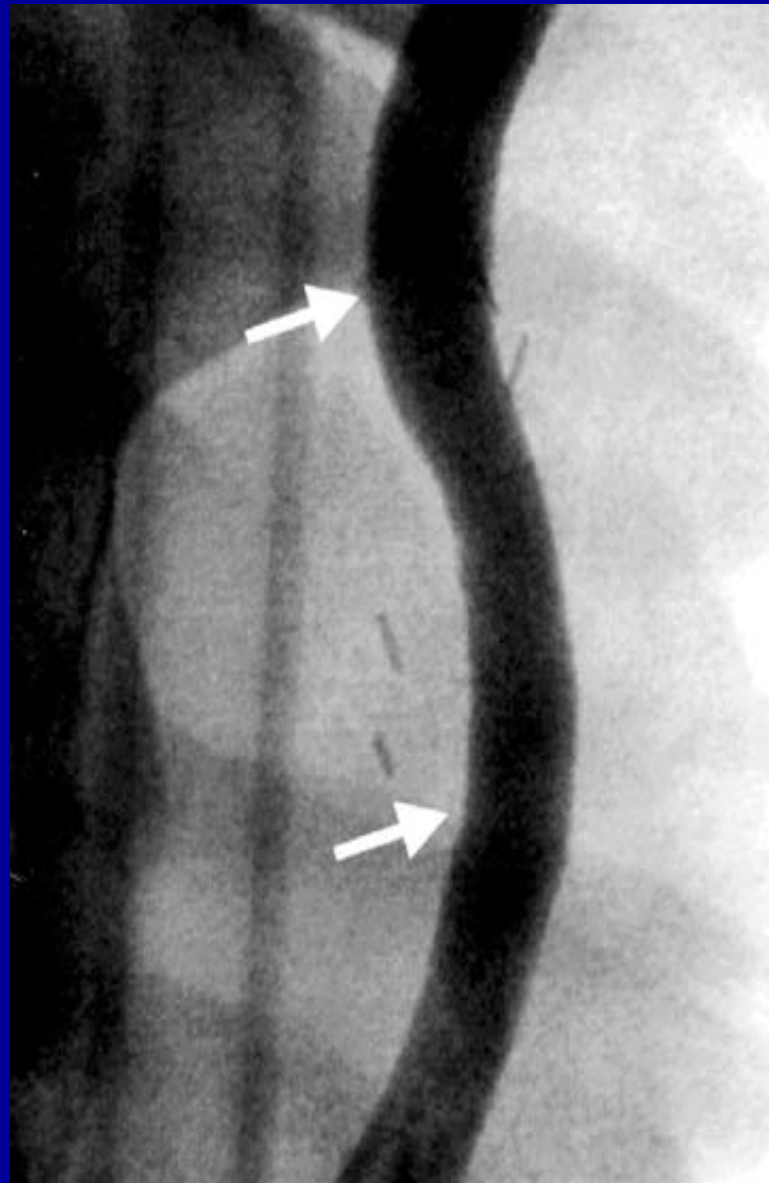
F VIII



CD14



Control

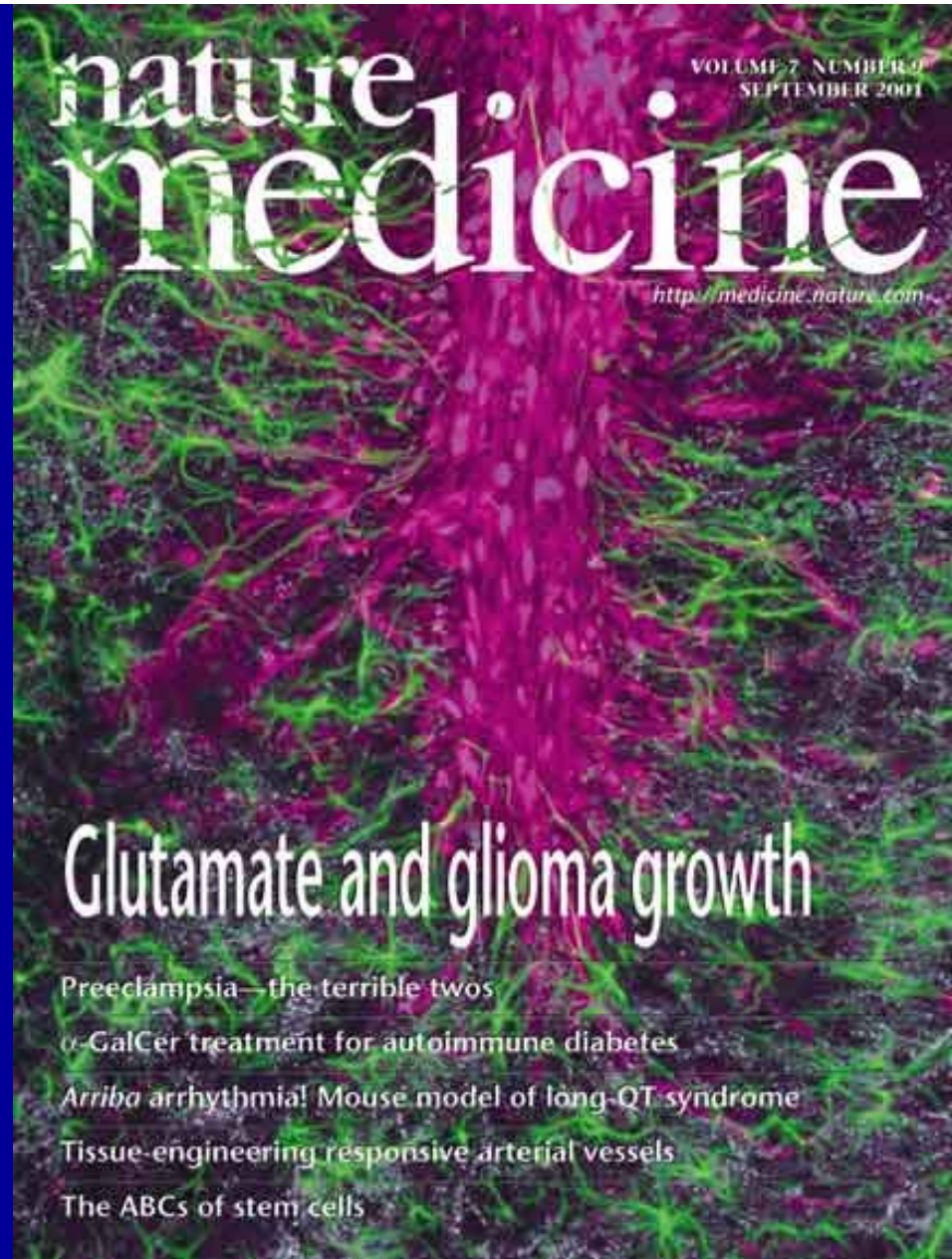


Engineered Artery



Native Artery

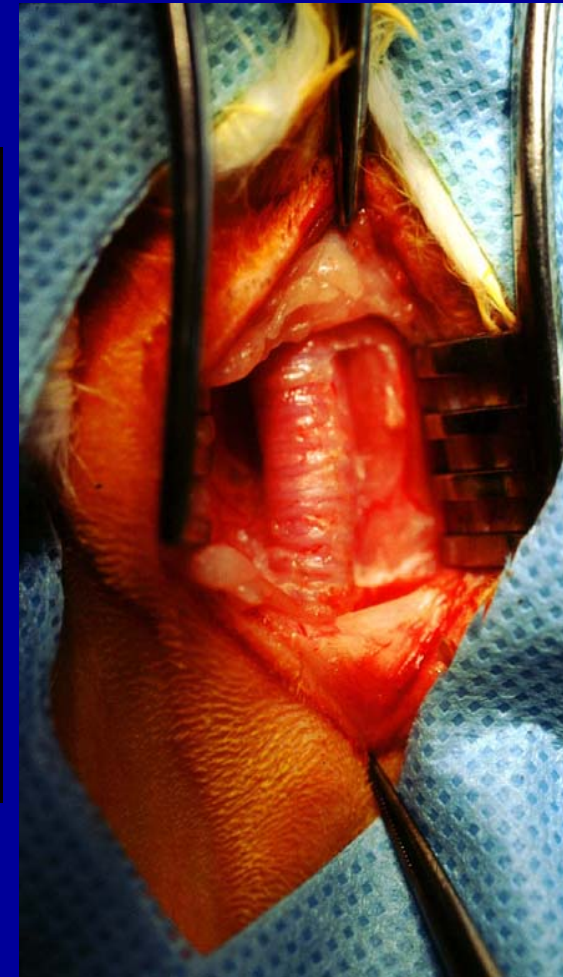
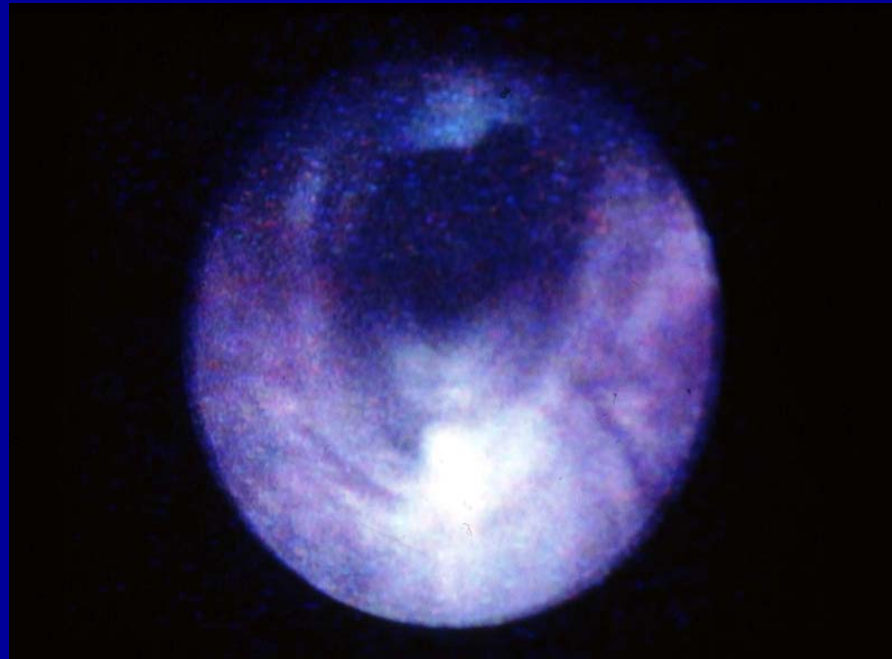




*S. Kaushal et al*

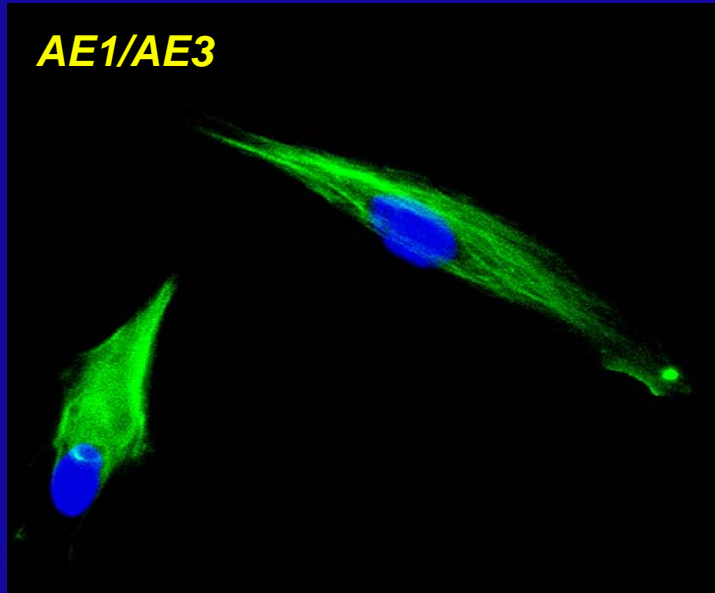


# Engineered Trachea

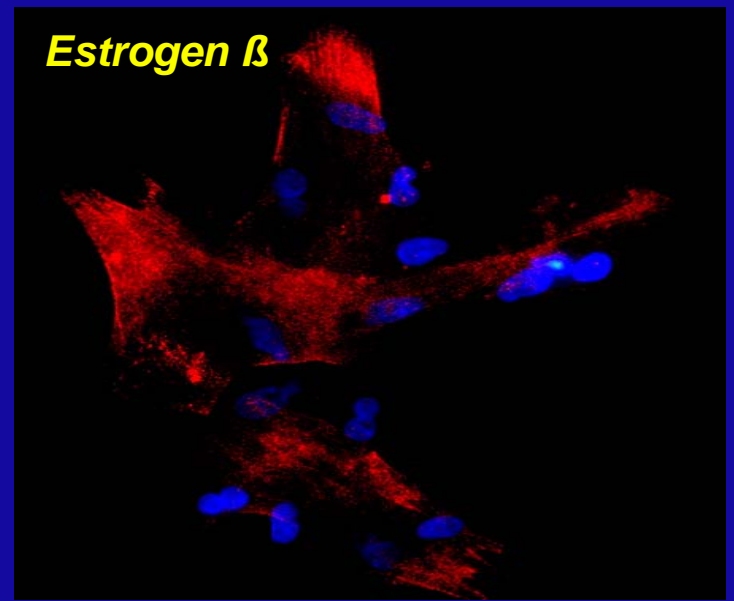


**Vaginal Epithelial  
Cells**

**AE1/AE3**

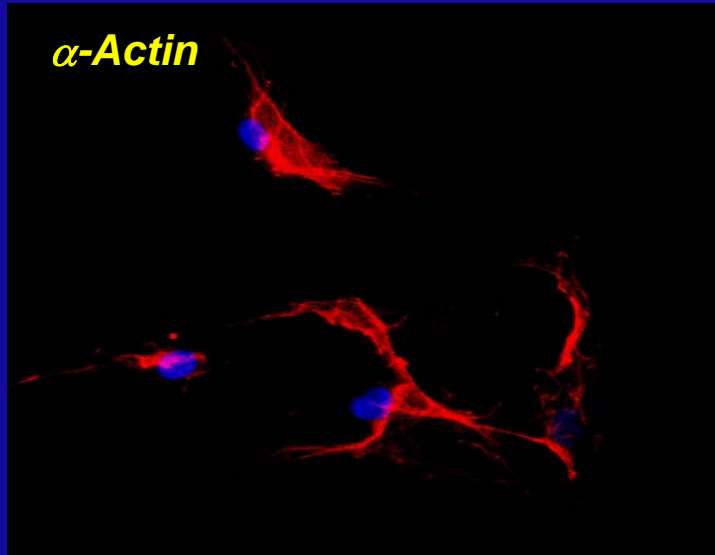


**Estrogen  $\beta$**

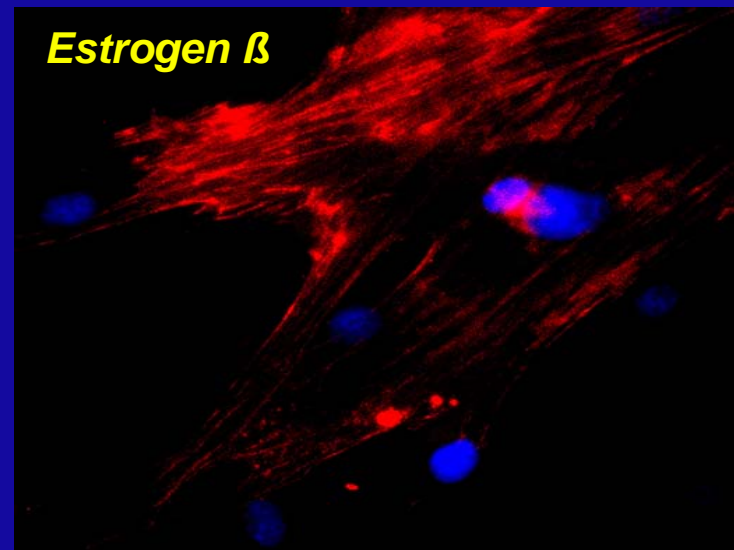


**Vaginal Smooth  
Muscle Cells**

**$\alpha$ -Actin**

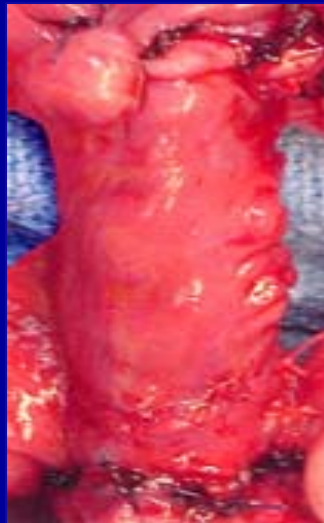


**Estrogen  $\beta$**



# Gross Examination

**1 Mo**



**3 Mo**

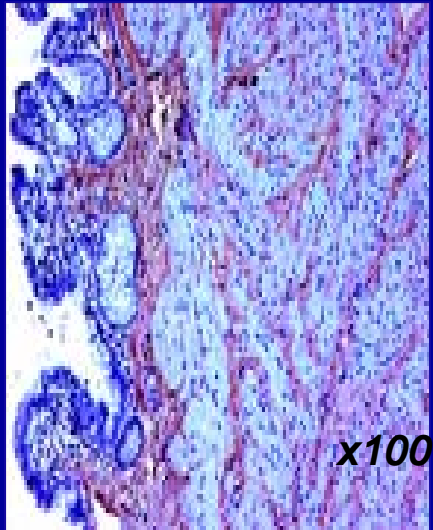


**6 Mo**

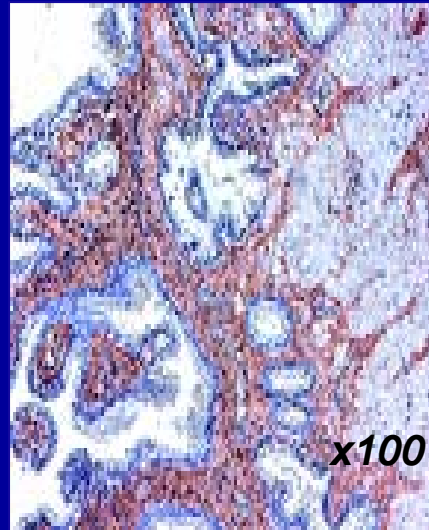


Engineered

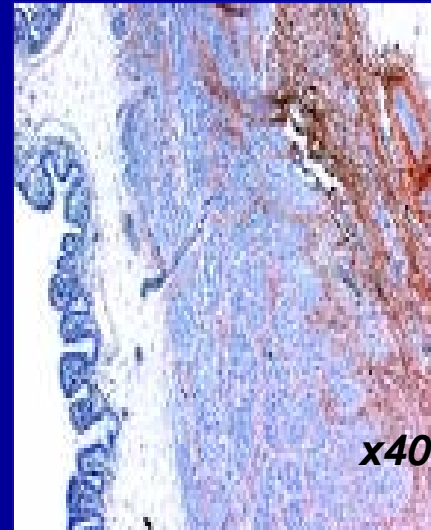
Collagen-I



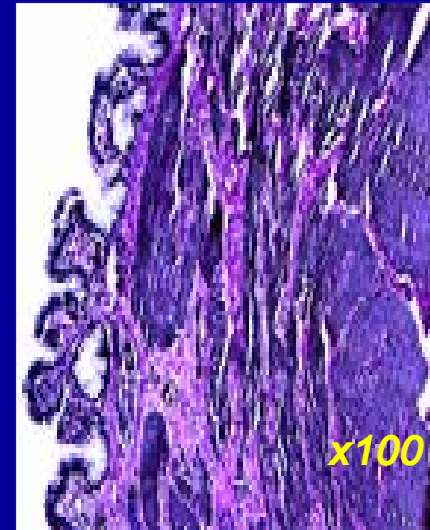
Collagen-II



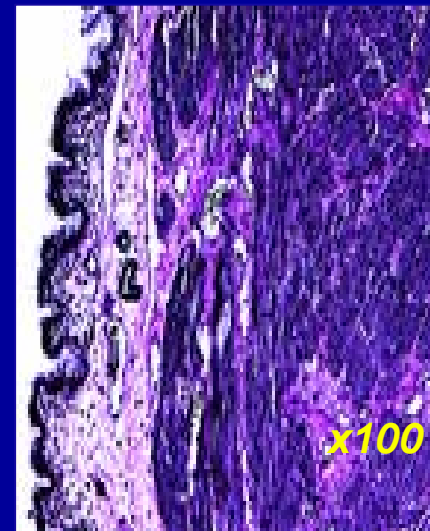
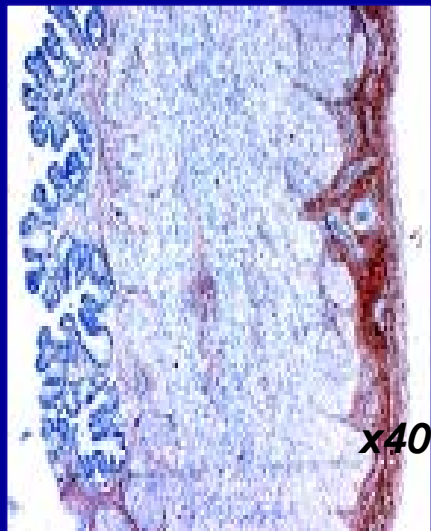
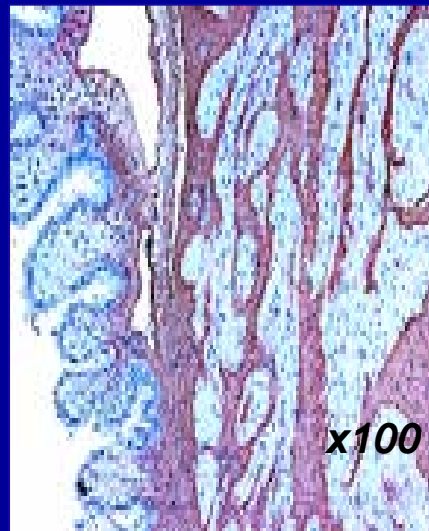
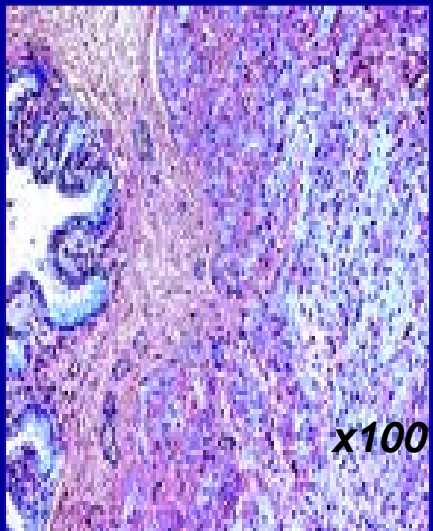
Collagen-III



Elastin



Normal





# Organ Bath Studies

**EFS**

**30 Hz**

**60 Hz**

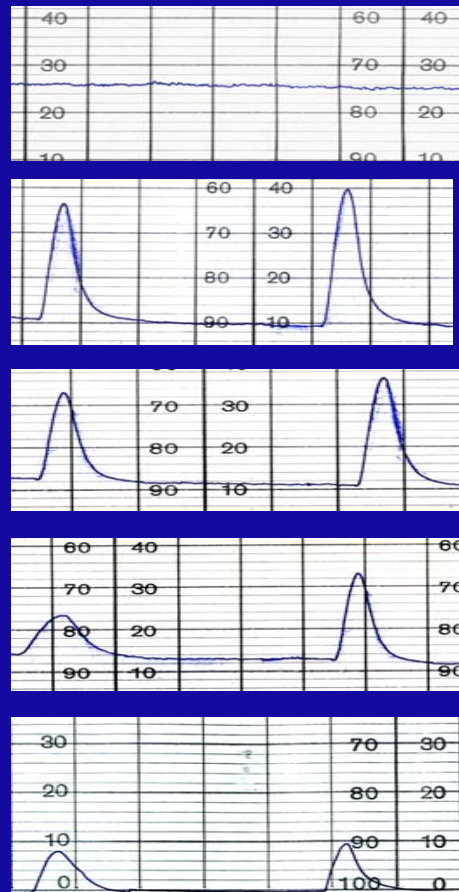
**Unseeded**

**NL Vagina**

**6 Months**

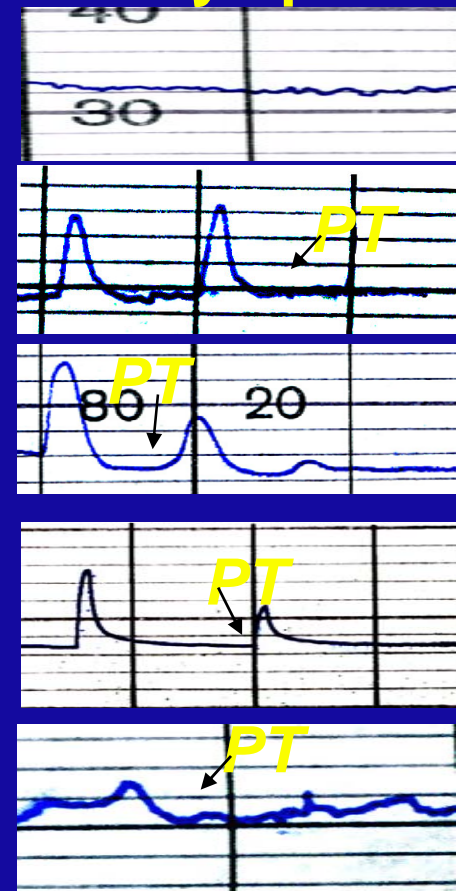
**3 Months**

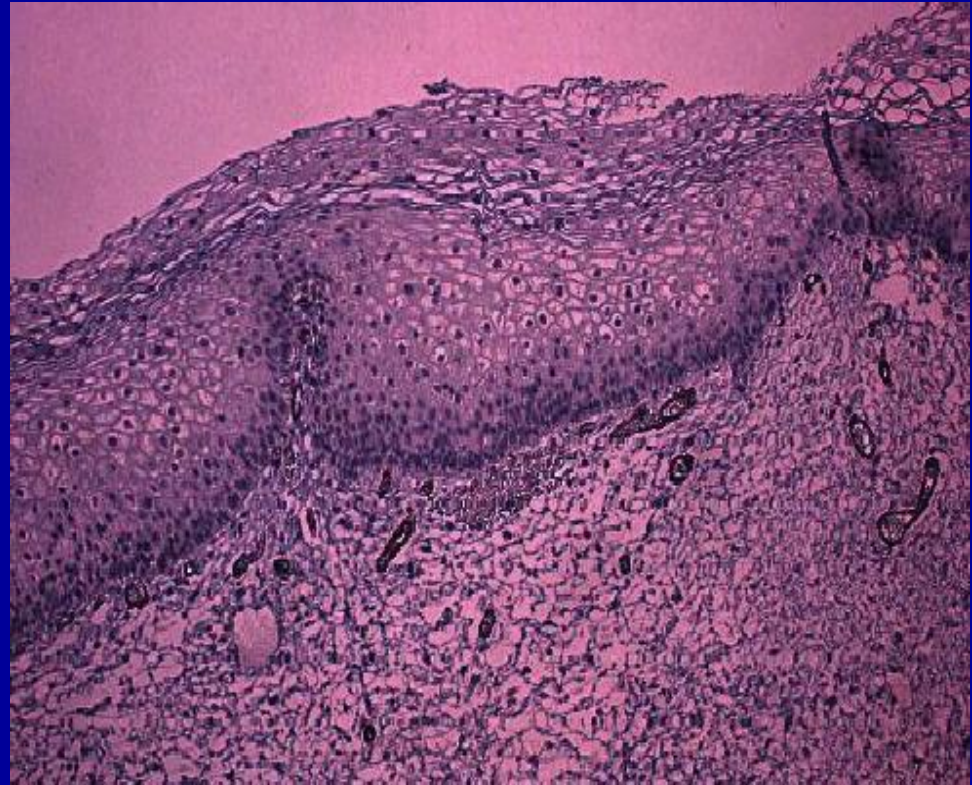
**1 Month**



**AR Stimulation**

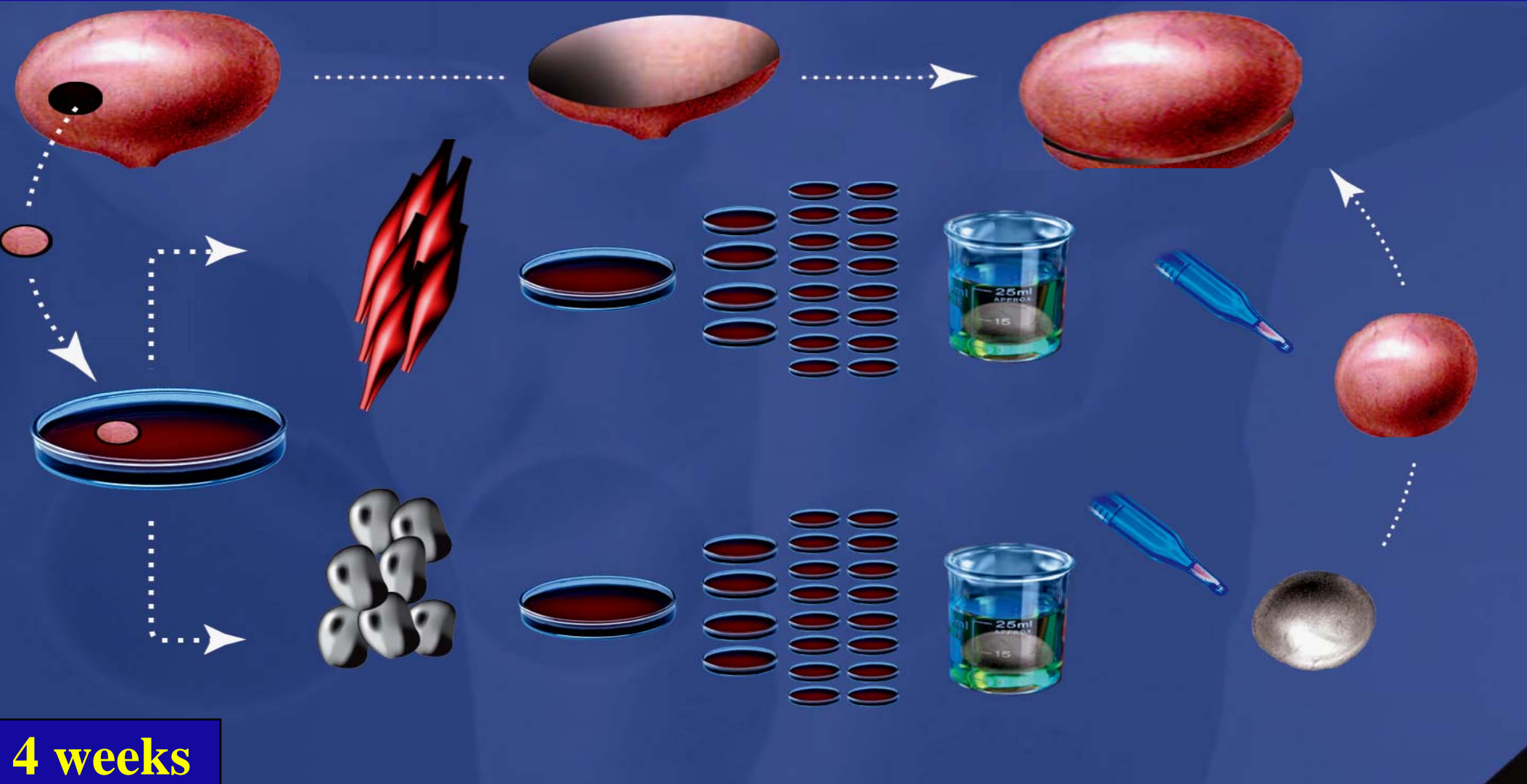
**Phenylephrine**





**Clinical Experience- 3 year follow-up in patients  
with engineered vaginas**

# Creation of the First Engineered Organ: Bladder





nature  
biotechnology

<http://biotech.nature.com>

VOLUME 17 NUMBER 2 • FEBRUARY 1999

**Artificial bladder  
takes shape**

**Transgenics make milk more digestible**

**Designer drug targets**

**Protein folding in practice**

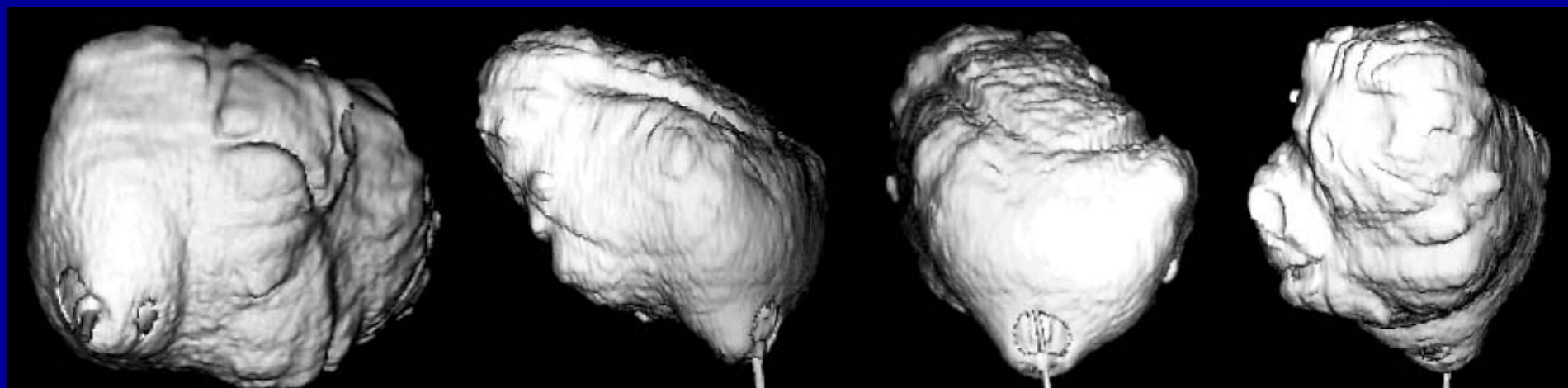
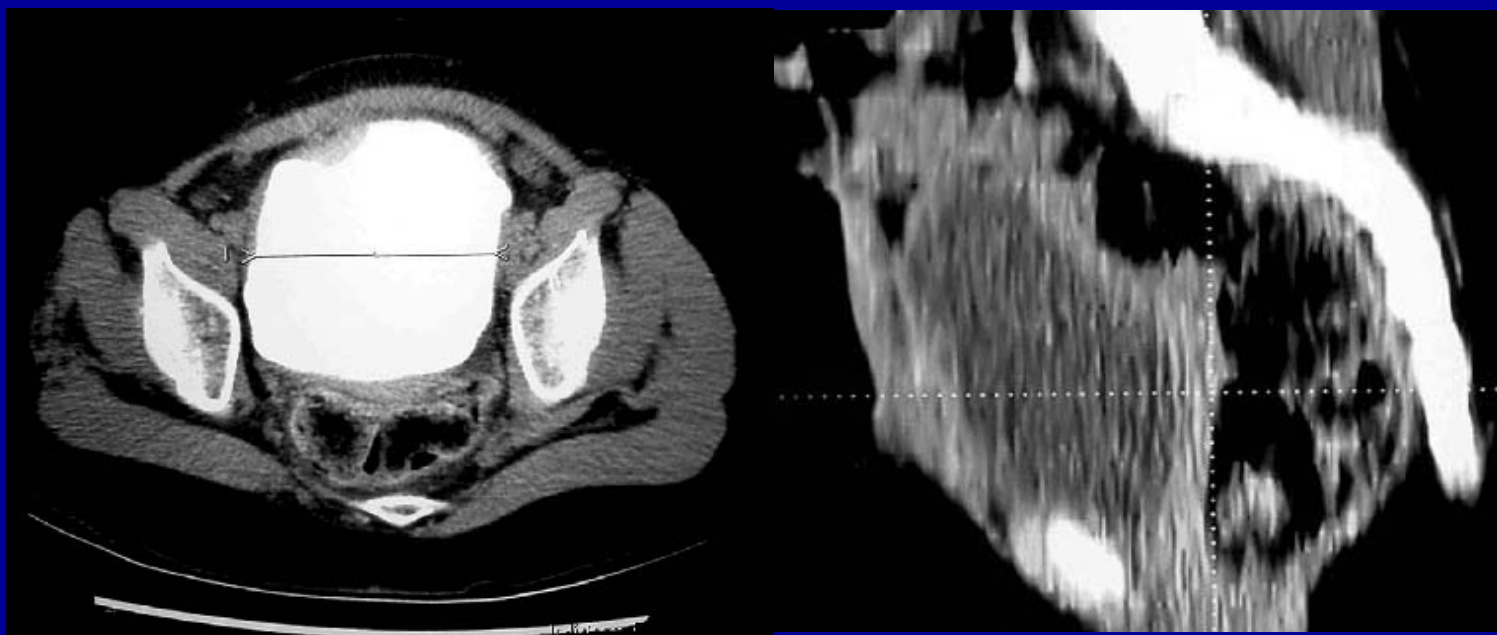
**Oberpenning et al, 1999**

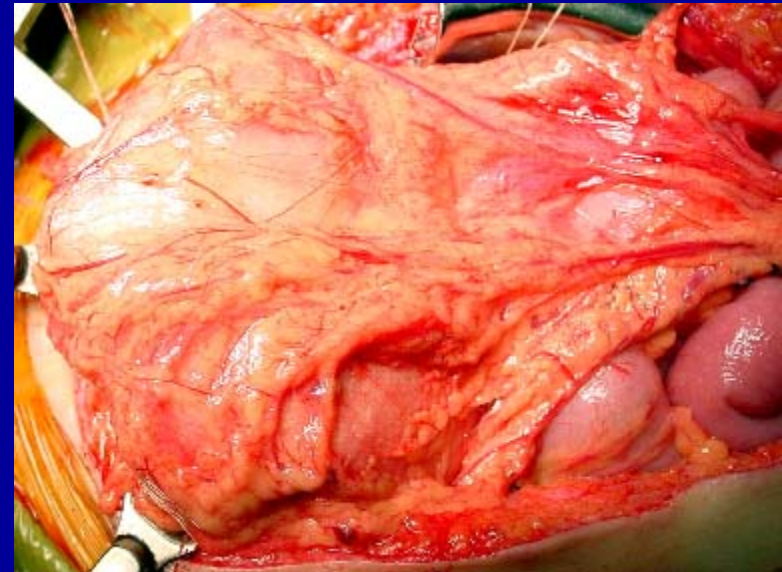
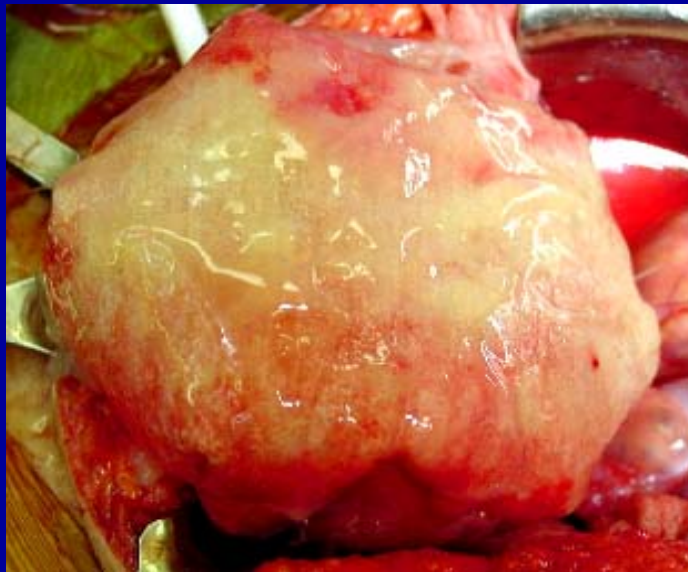
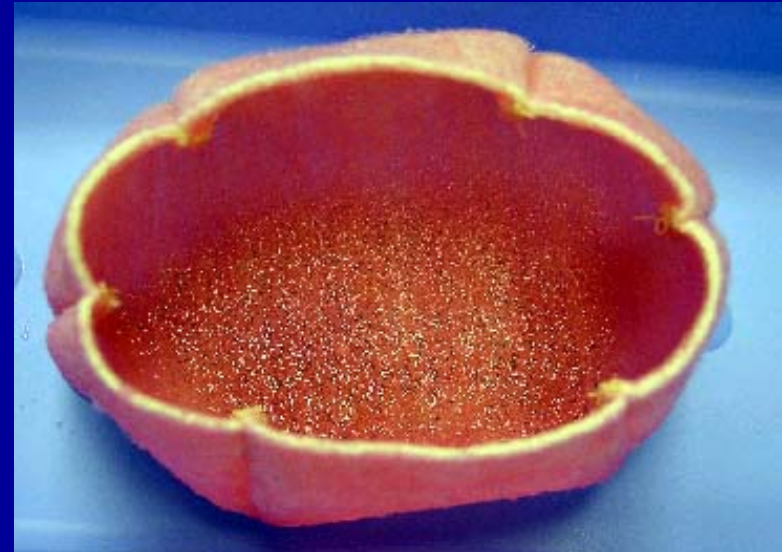


# **Clinical Studies**

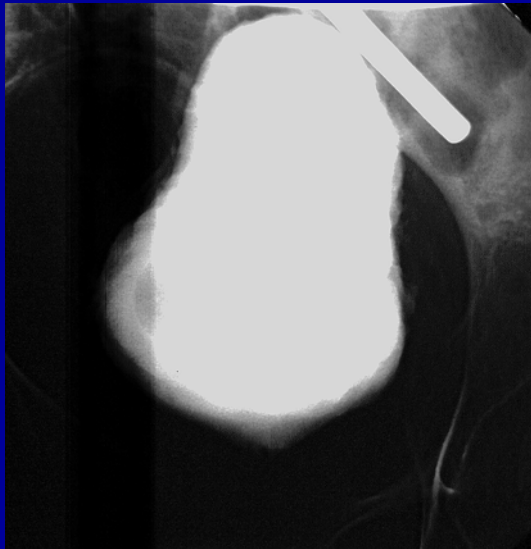
**Patients with high pressure /low capacity bladders**

**All failed medical therapy and were considered candidates for bladder reconstruction**

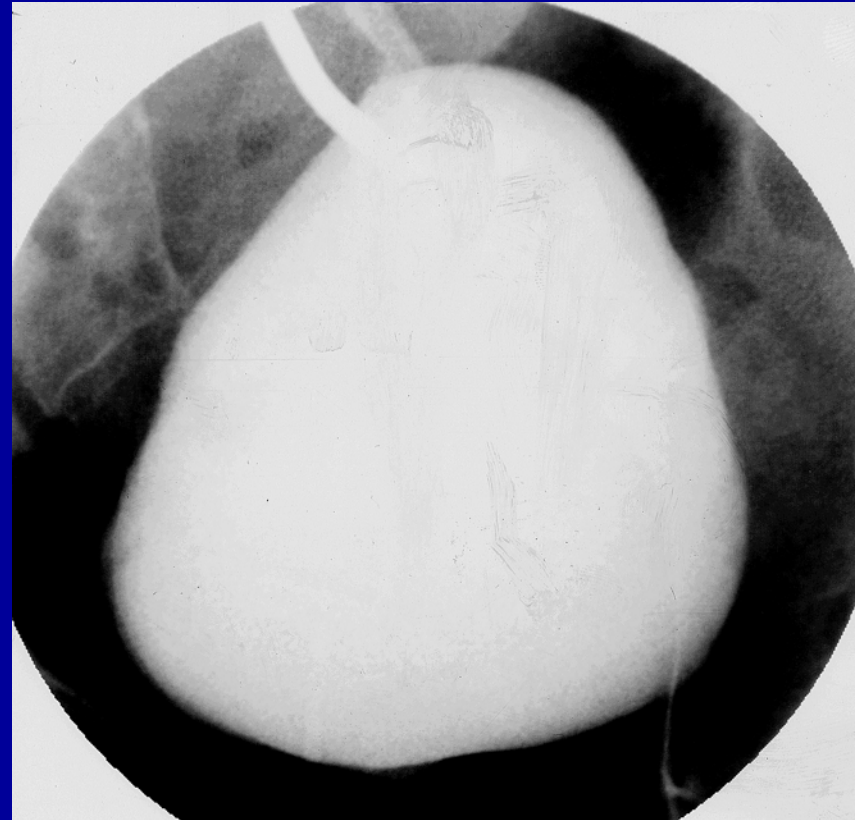




**Pre-Op**



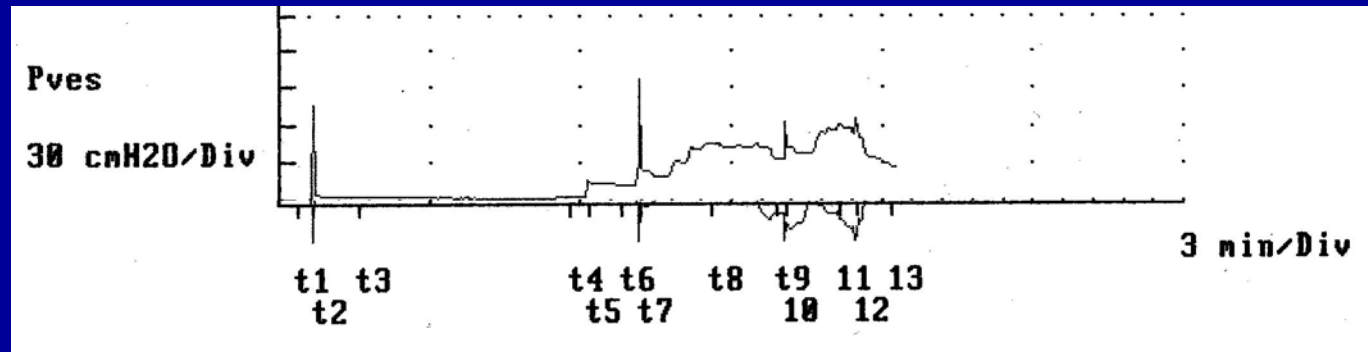
**Post-Op (6 Mo.)**



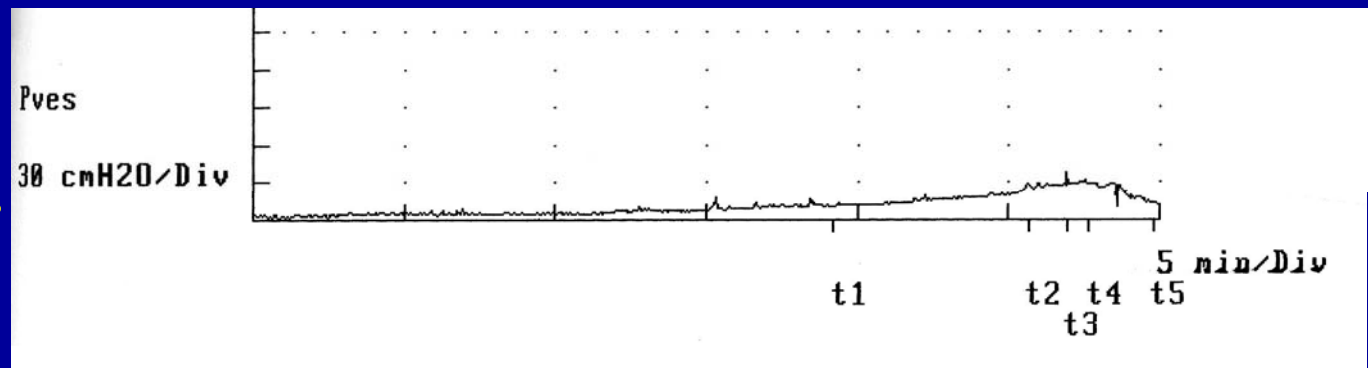


# Urodynamic Studies

Pre-Op.



Post-Op.





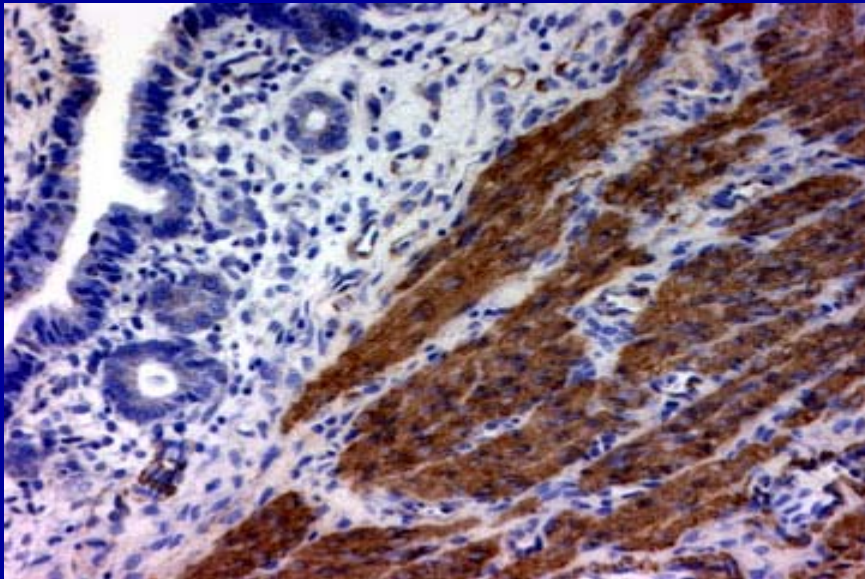
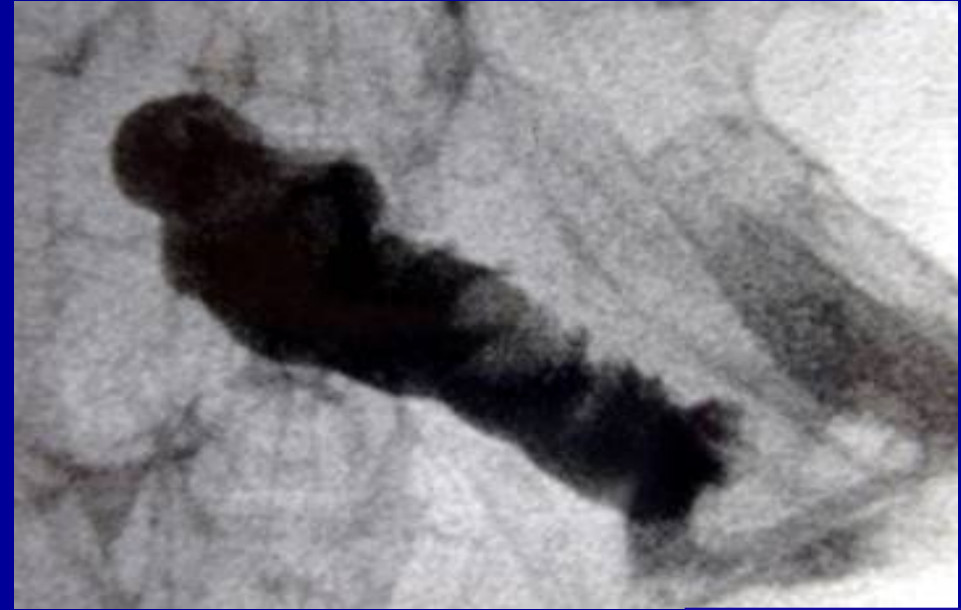
## Clinical Experience

3 protocols

5 year follow-up

*The Lancet, April 06*

# UTERUS



*kD*      NL      CS

61 —

49 —

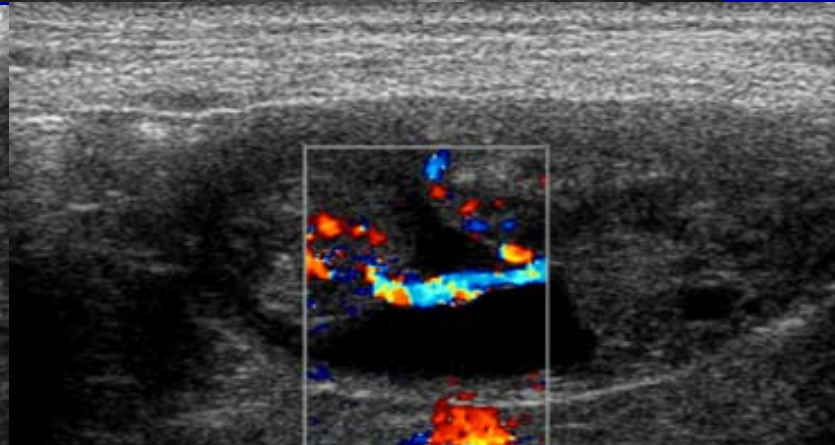
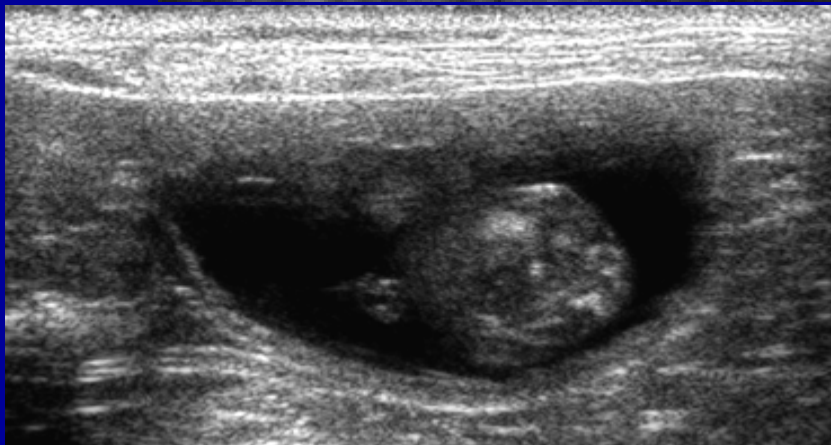
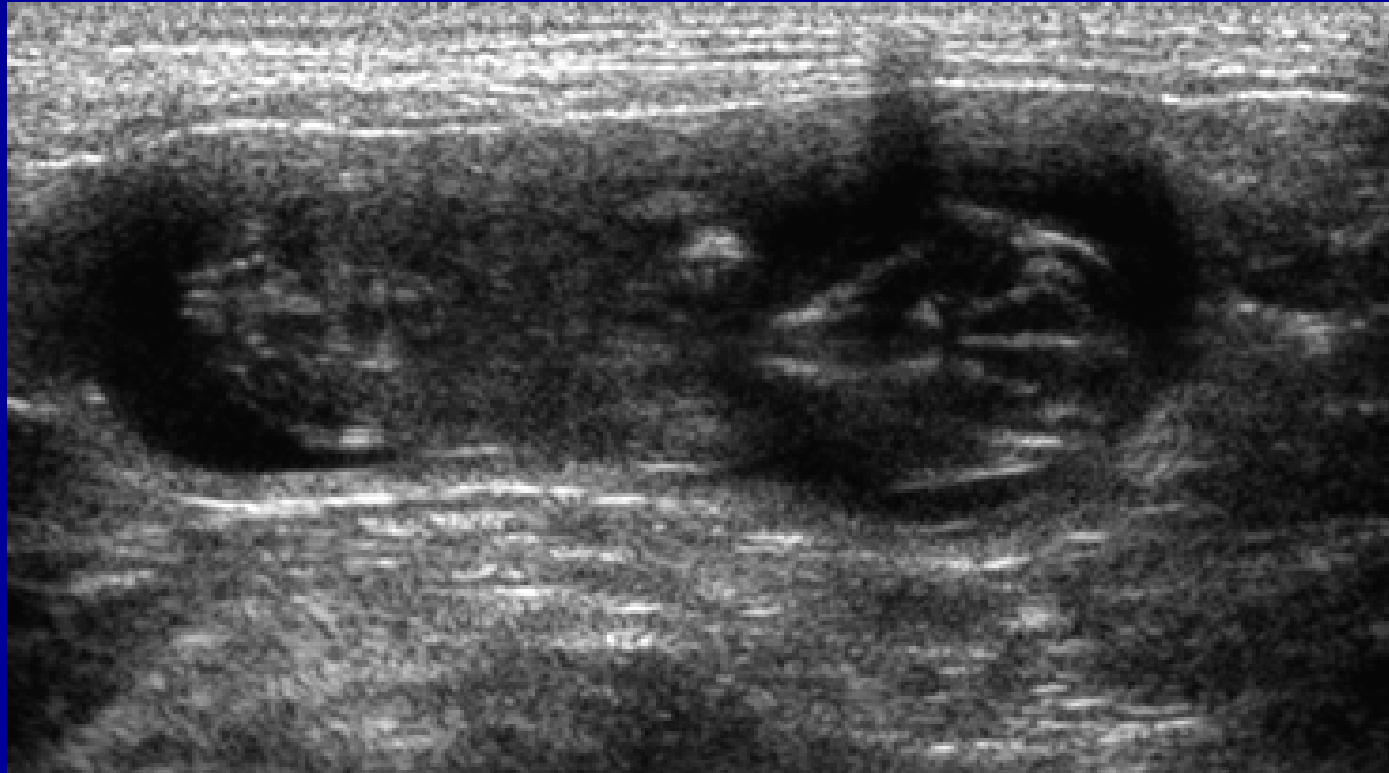


Estrogen receptor B



# Fetus in Tissue Engineered Uterus

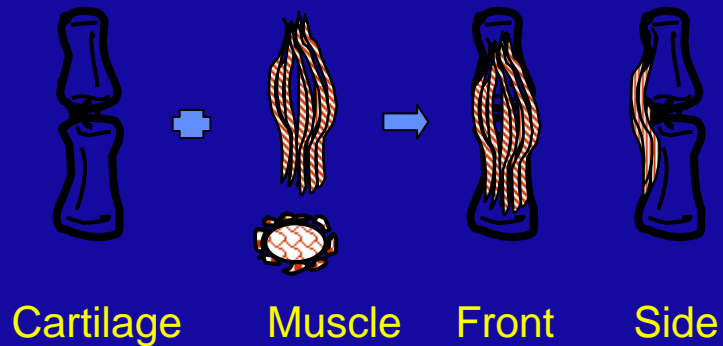
## *Near-term*



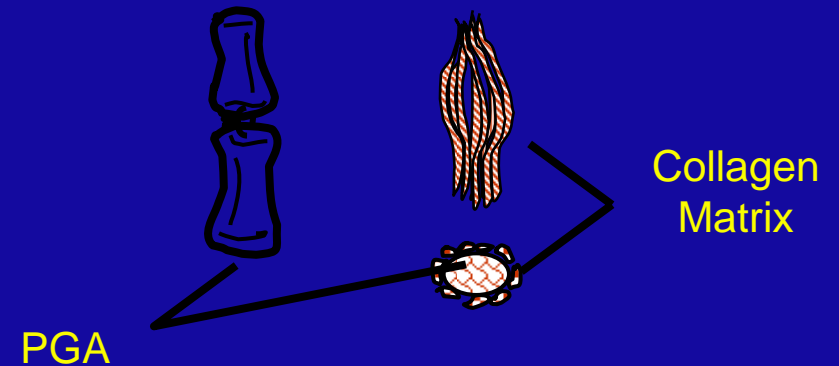


# Engineering of the Digit

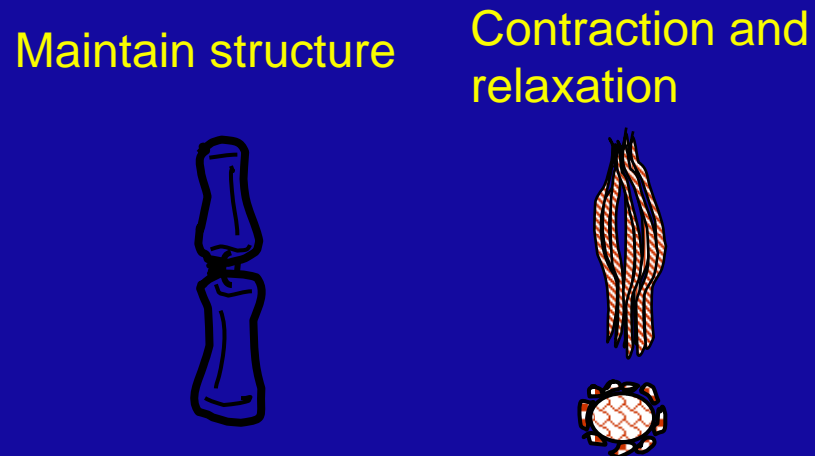
## Scaffold Configuration for Digits



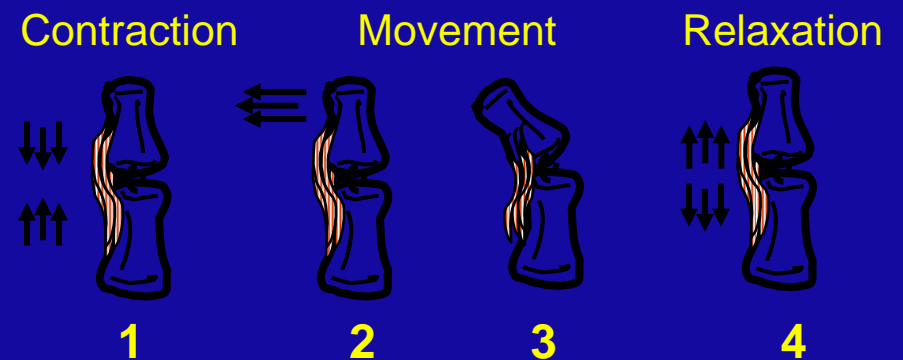
## Scaffold Composition



## Functional Components



## Muscle Function and Digit Movement



# Engineered Digit

## *Cartilage and muscle composite tissue*

C a r t i l a g e

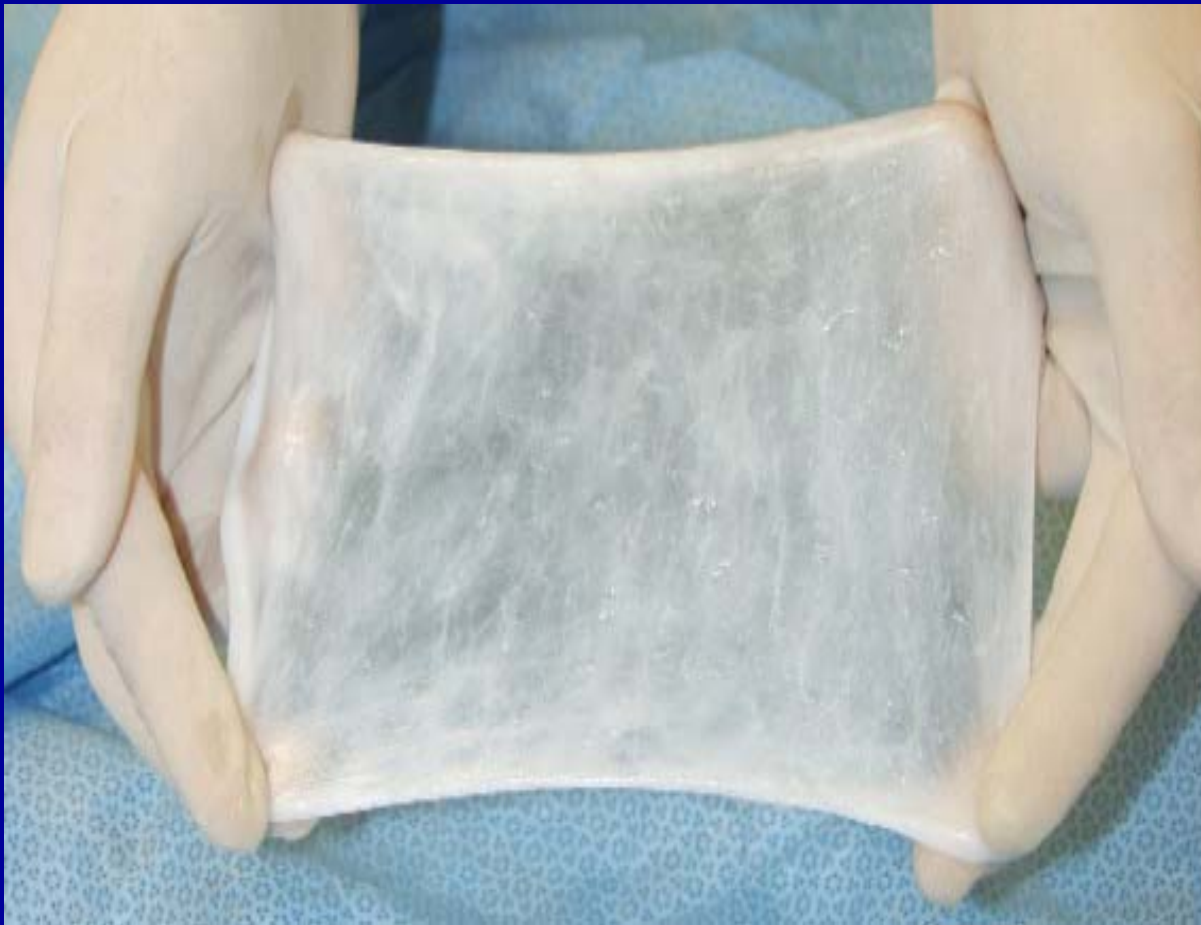


M u s c l e



# Steve Badylak, MD, PhD, DVM

## Material-Induced Regeneration



Commercially  
available product

FDA approved

## Fingertip Regeneration in 78-year-old man



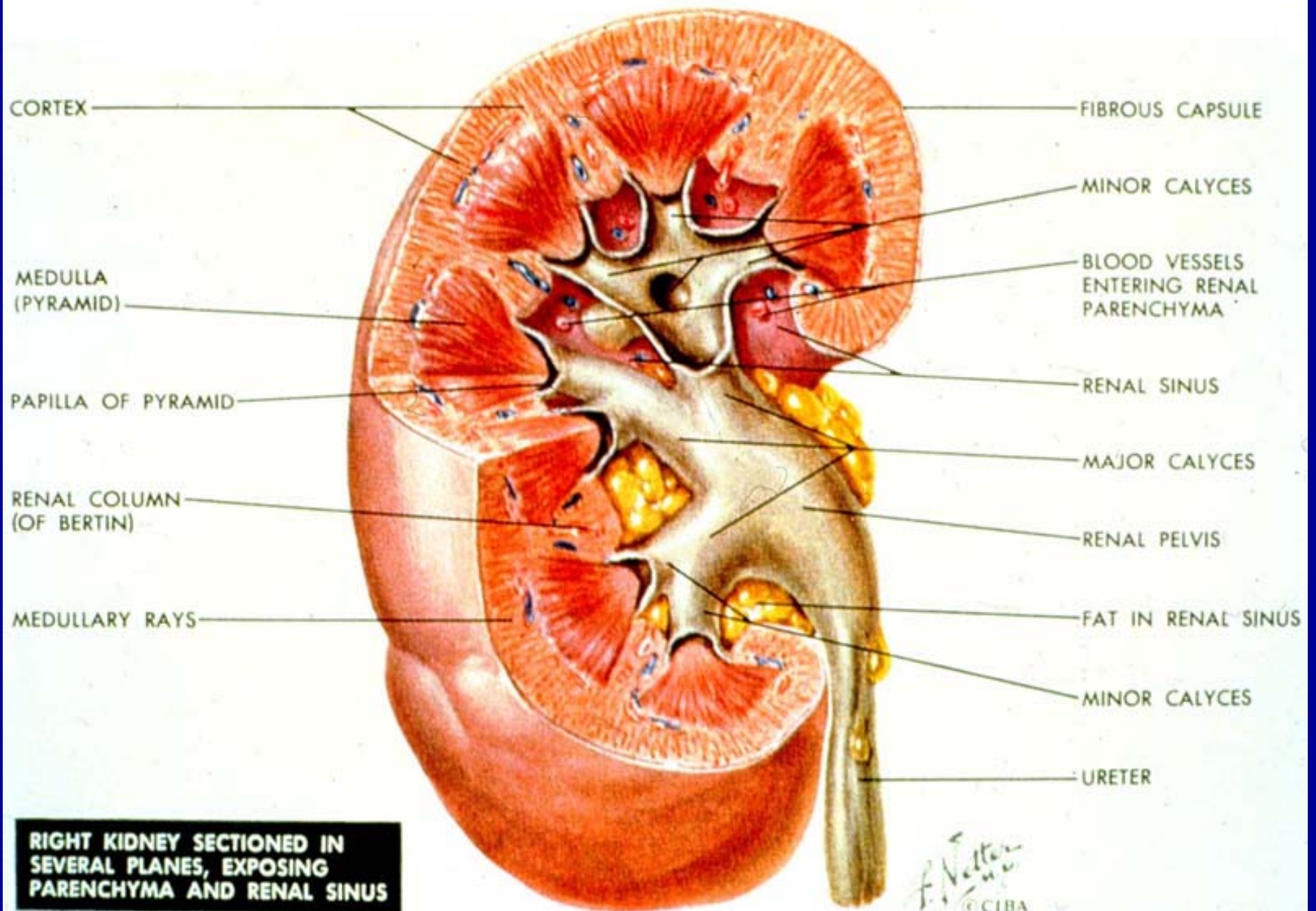


# Fingertip Regeneration in 78-year-old man

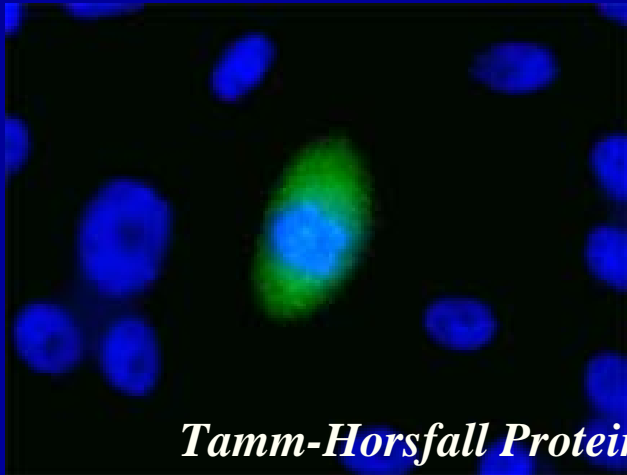


# Engineering of Ears

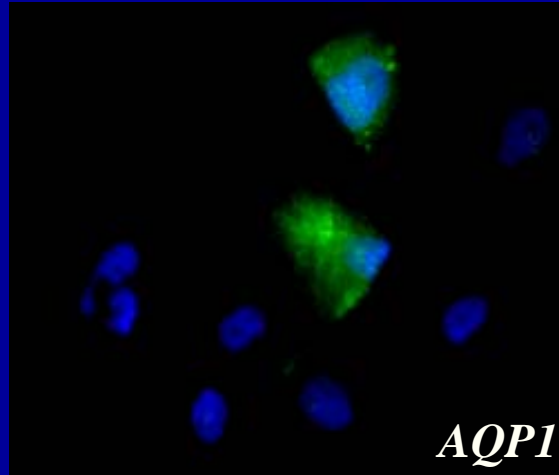




# Renal Cells



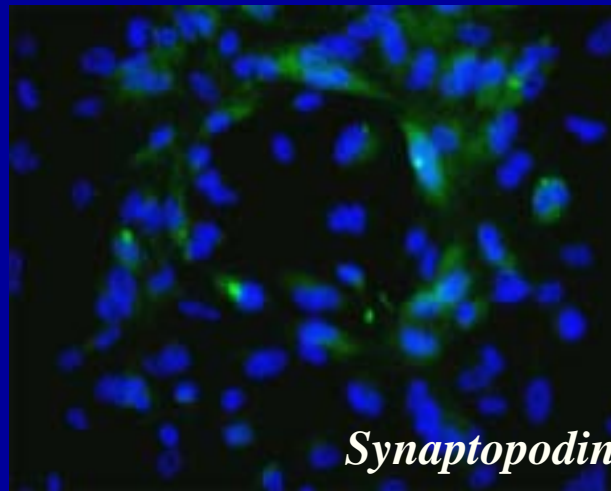
*Tamm-Horsfall Protein*



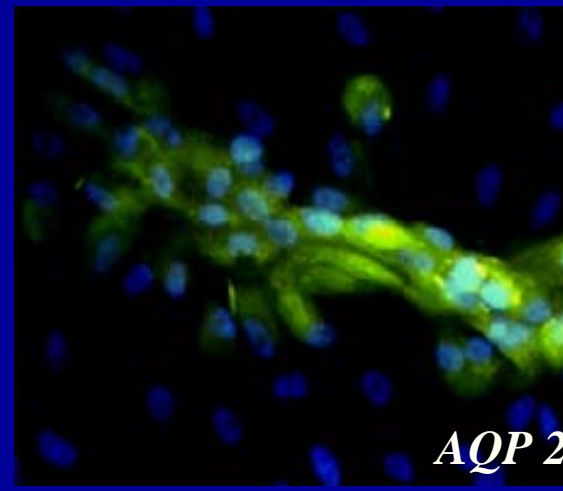
*AQP1*



*Von Willebrand factor*



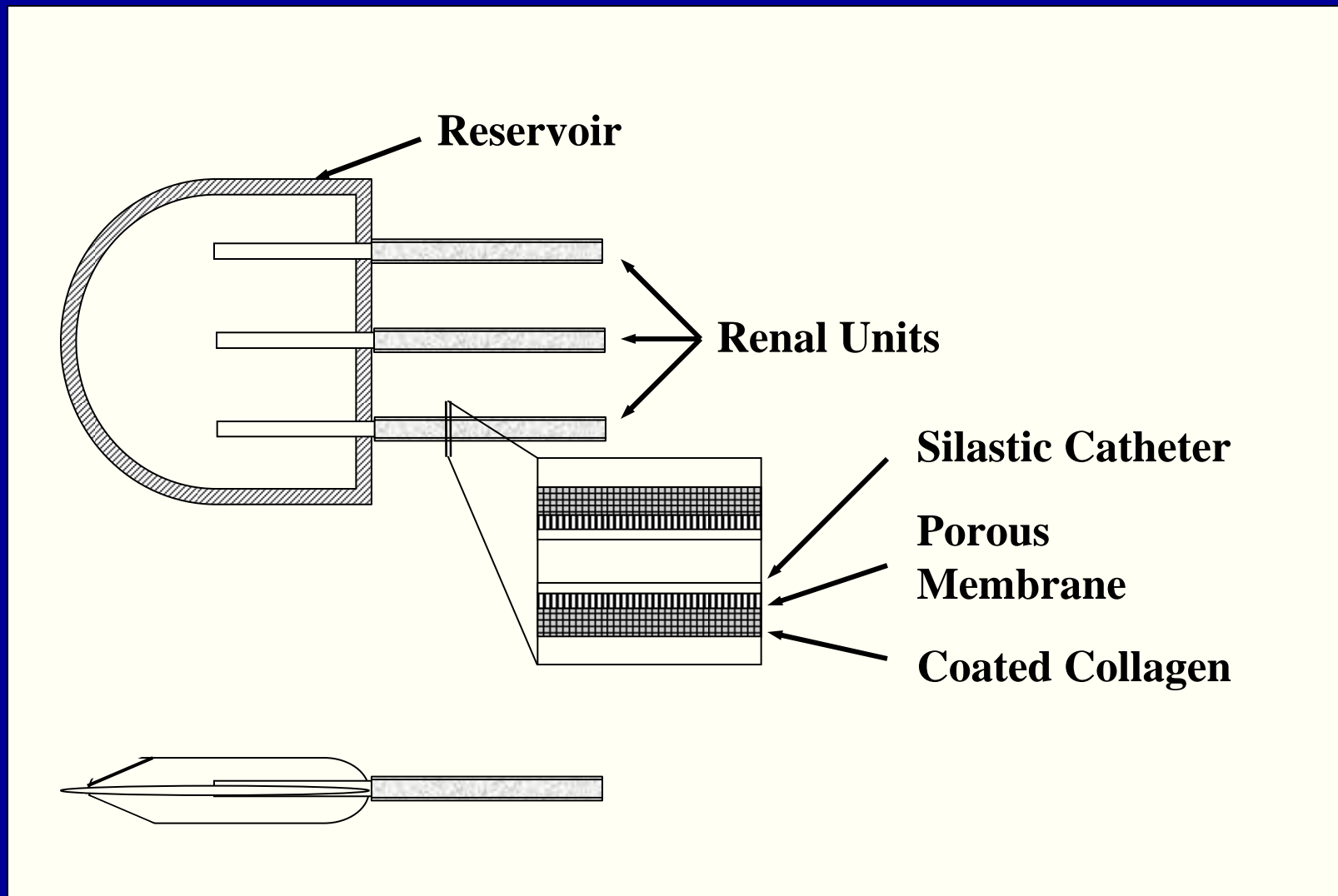
*Synaptopodin*



*AQP 2*



# Renal Device



# Retrieved Renal Units

**Cloned Cells**



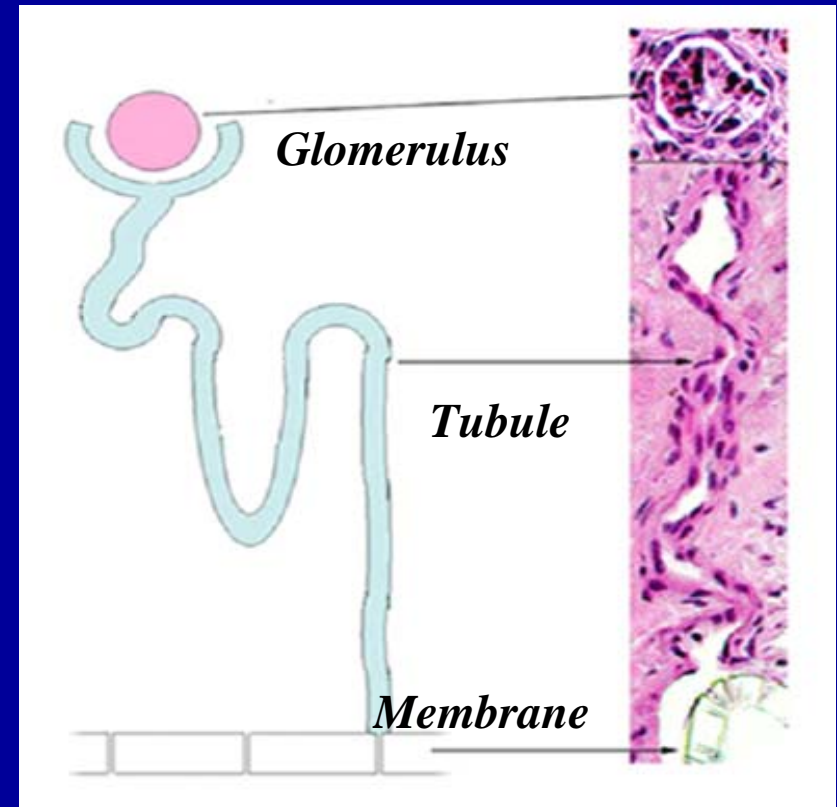
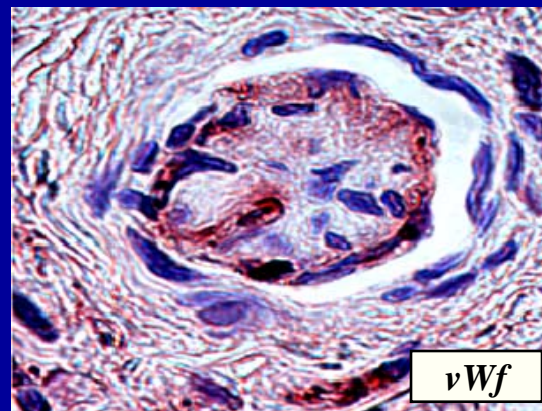
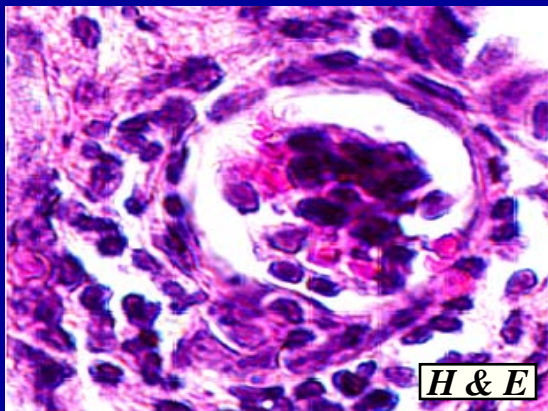
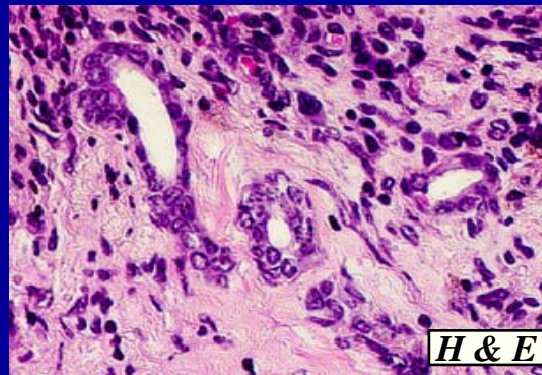
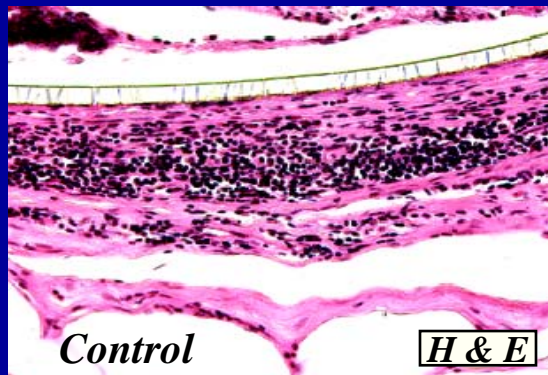
**Allogeneic Cells**



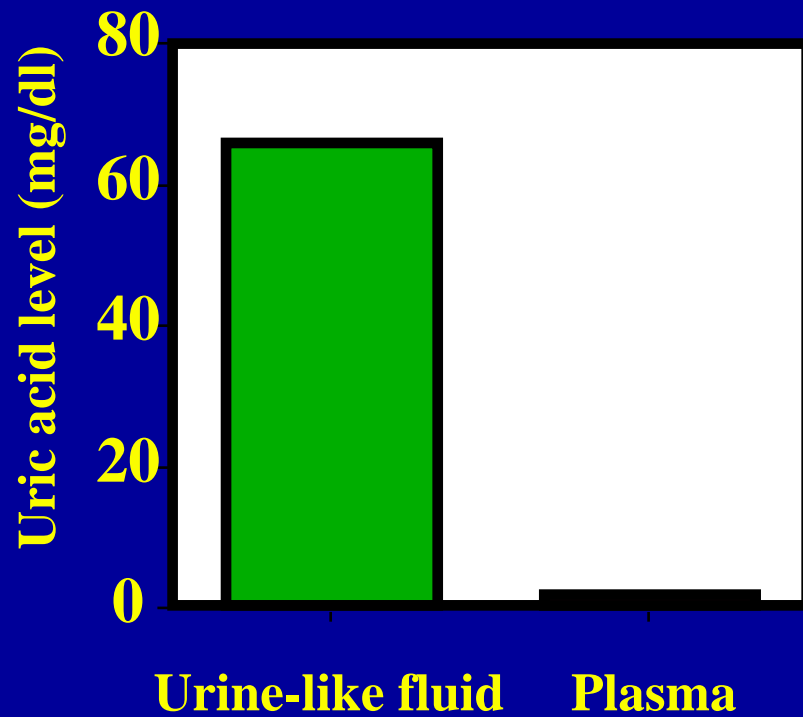
**Unseeded**



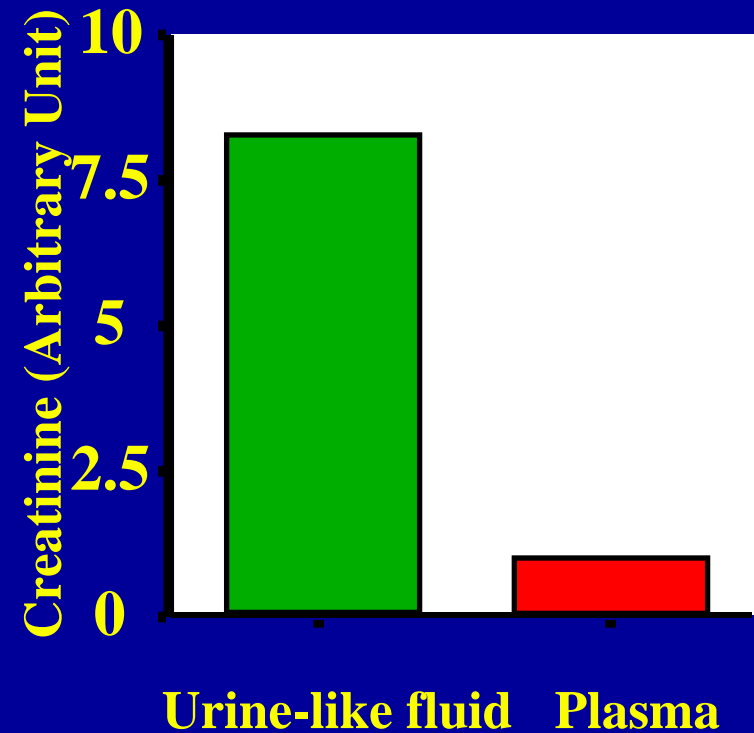
# Retrieved Renal Tissues



## Uric Acid



## Creatinine

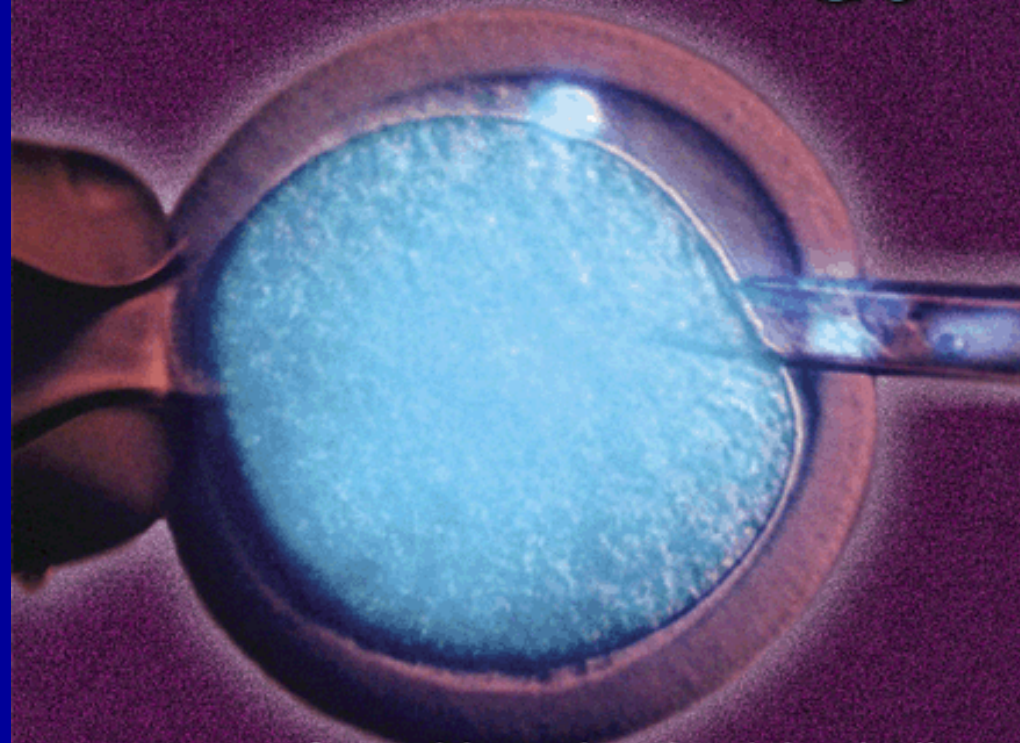




# nature biotechnology

VOLUME 20 • NO 7 • JULY 2002

<http://biotech.nature.com>



**Successful transplantation of cloned cells**

Genome shuffling for industrial microbes

Ribozymes throw light on drug targets

Lactobacilli fight tooth decay

# CELL THERAPY

# **Injectable Cells for Therapy**

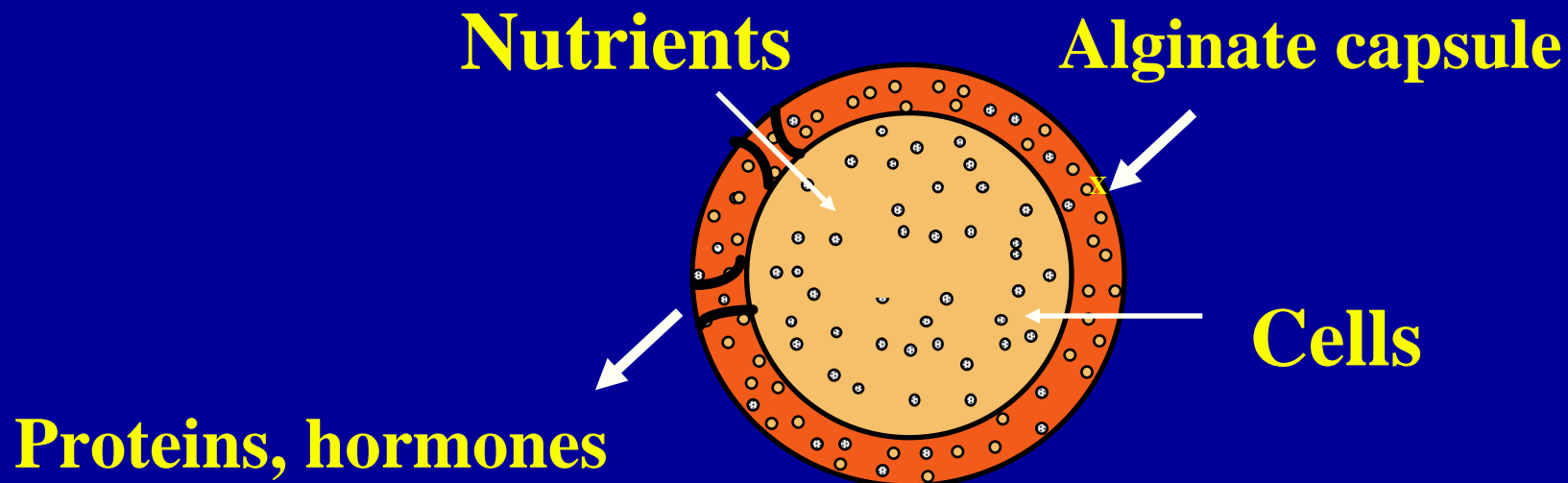
**Cartilage cells : FDA phase II and III  
multicenter clinical trials, 110 patients, 10  
centers, 5 year follow-up**

**Muscle cells: Phase 1 FDA trial, 32 patients,  
single Injection, 80% success at 3 and 12  
months, 5 year follow-up**

## Empty Microcapsules



## Encapsulated Cells



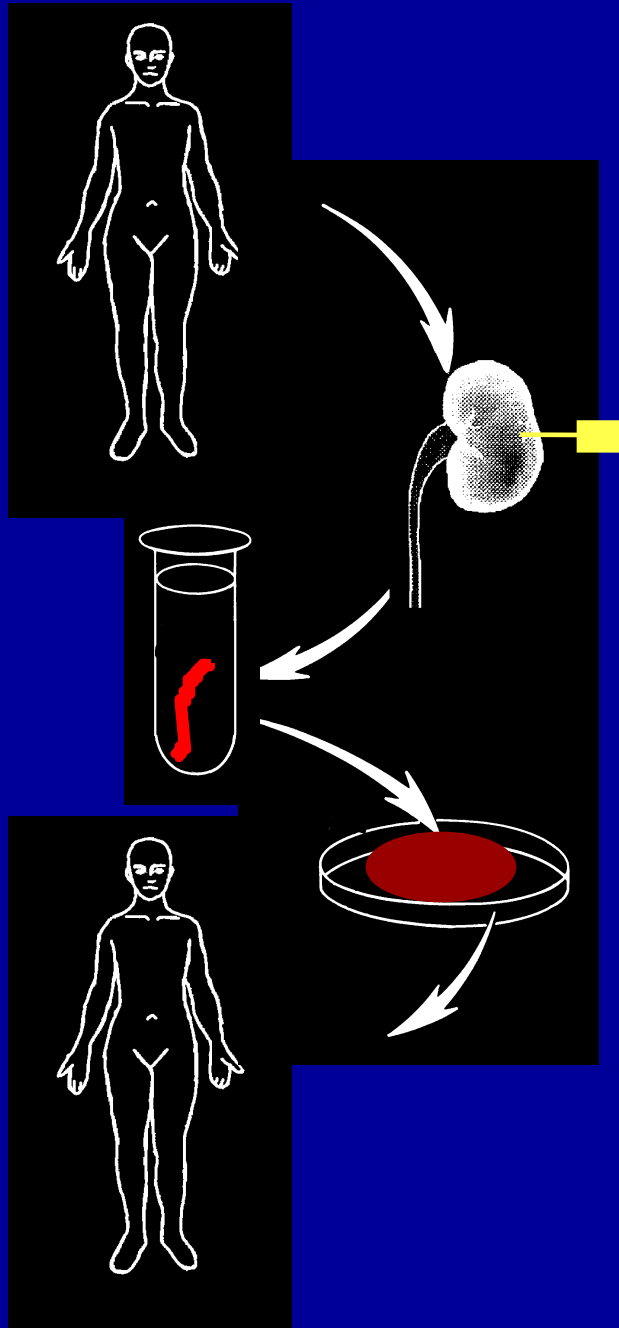




## Applications for Engineered Cells

**Tumor therapy**  
**Endostatin**  
**Others**

**Excretion of proteins/ hormones**  
**Menopause (estrogen)**  
**Diabetes (insulin)**  
**Parkinson's (L-Dopa)**  
**Testosterone**

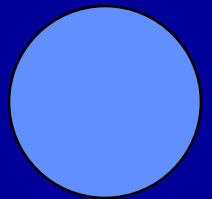


# Stem Cells

**A stem cell can become any cell and it can create any tissue or organ**

**Only 2 pluripotent stem cell types described to date:**

- Embryonic stem cells**
- Adult bone marrow stem cells**



**Pluripotent stem cells: only 2 identified to date**

**Embryonic stem cells**

**Pro: very high replicative potential**

**Con: Malignant potential, issues with rejection, ethical issues**

**Adult bone marrow stem cells:**

**Pro: low malignant potential, can be used without rejection**

**Con: very low replicative potential**



**Amniocentesis: amniotic fluid that bathes the fetus in the womb during pregnancy**

**Placenta: the tissue in the womb that houses the baby**

**Amniotic fluid and placental tissue: Possible source for stem cells?**

## Conclusions

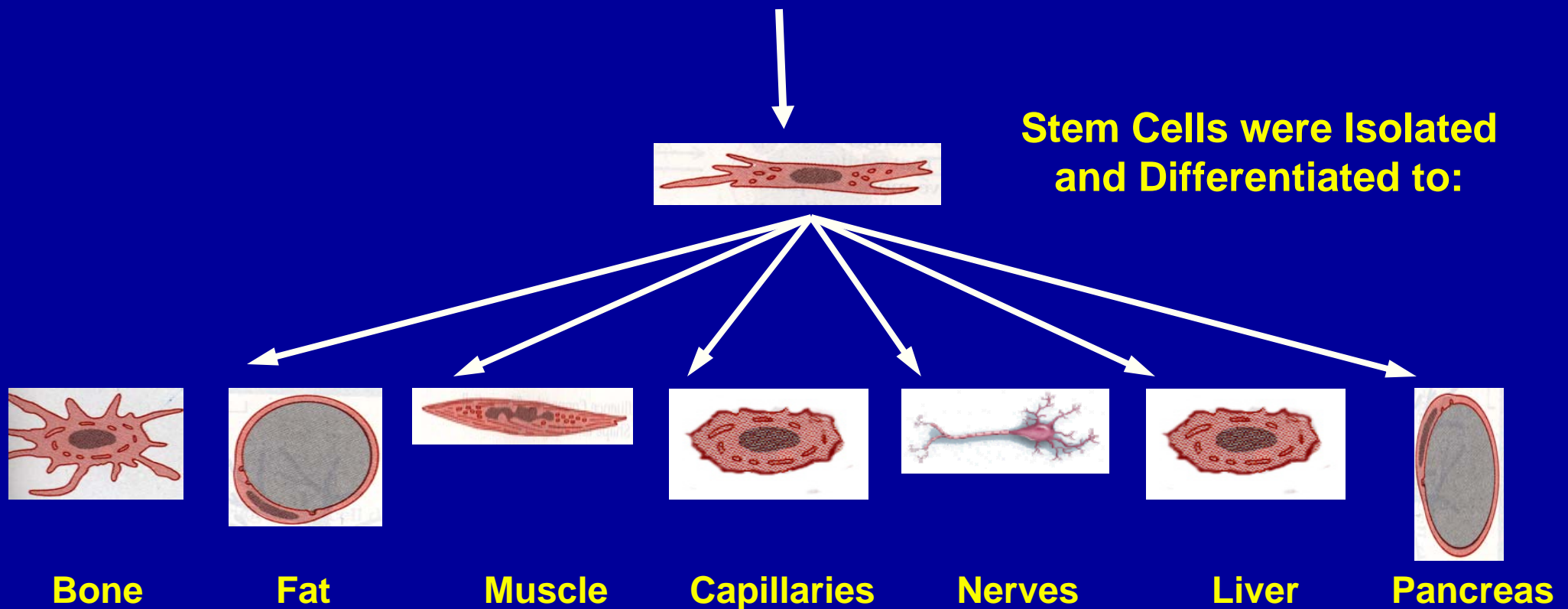
**A new cell class is described, derived from amniotic fluid and placental tissue obtained during pregnancy or at the time of birth.**

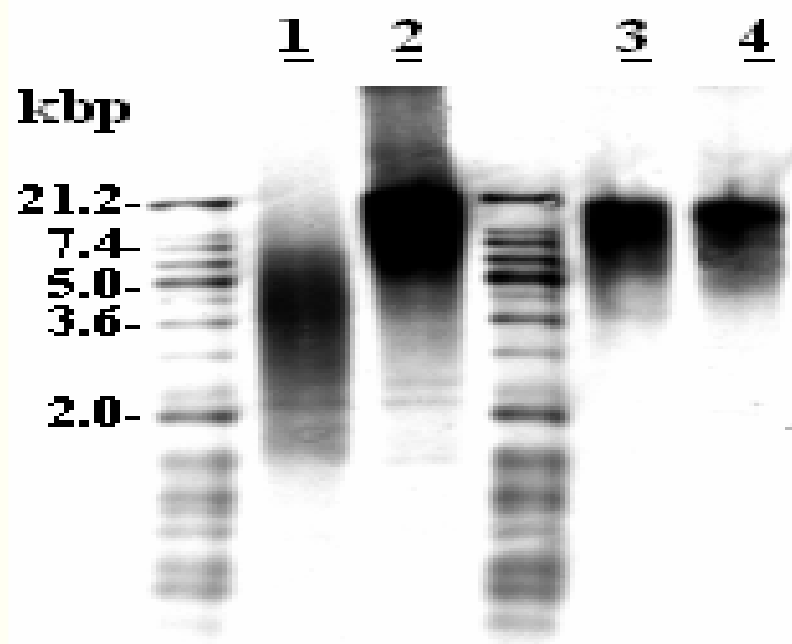
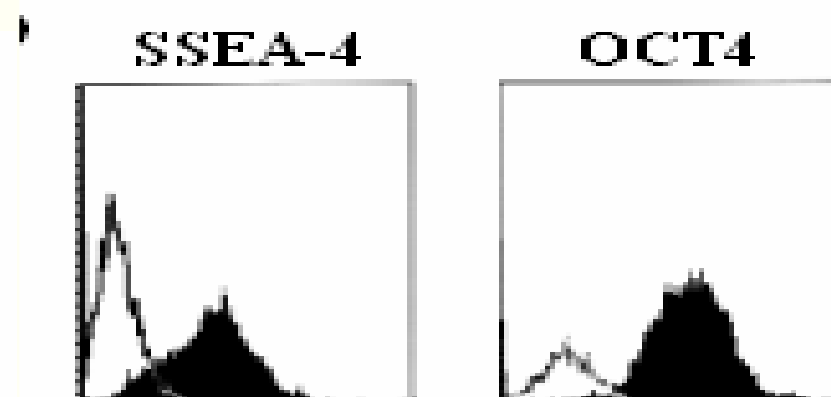
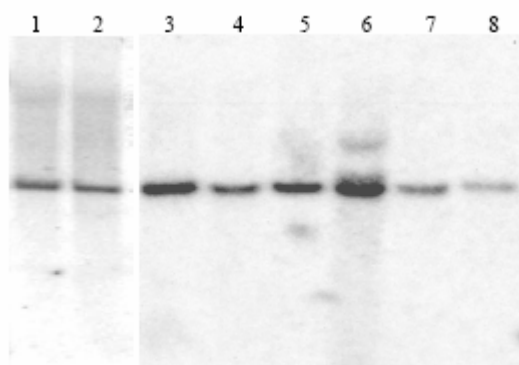
**This system avoids the malignant potential and ethical concerns surrounding the use of embryonic stem cells**

**The stem cells can be rapidly expanded to large quantities sufficient for clinical translation, thus avoiding the limitations of adult bone marrow stem cells,**

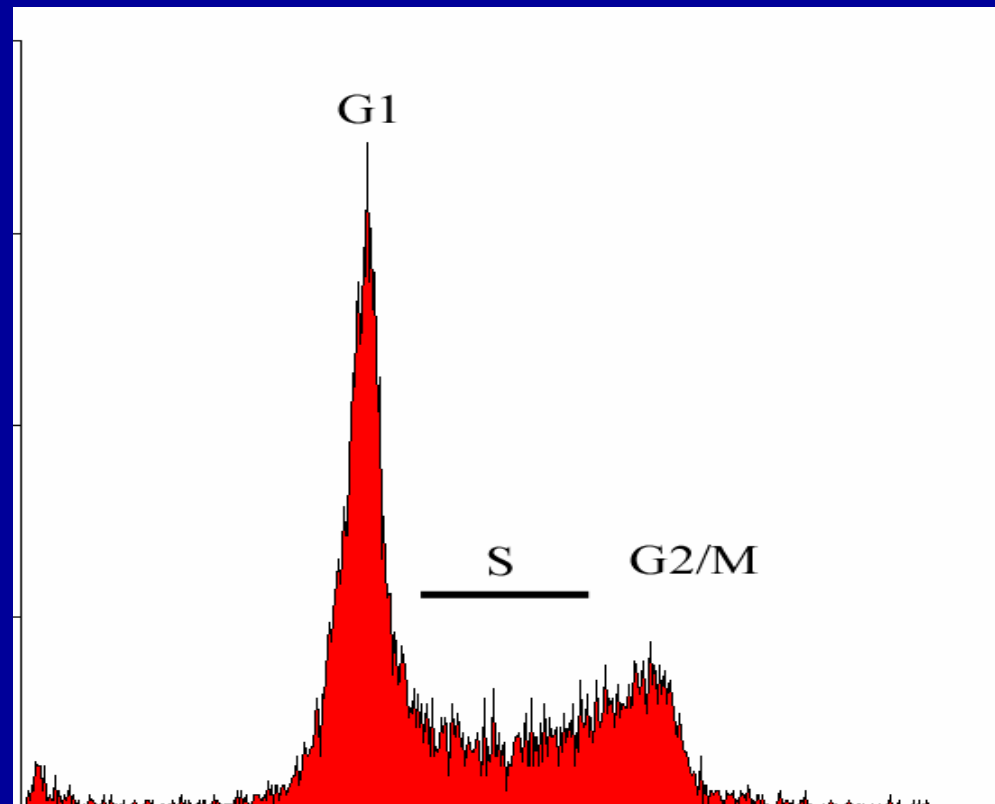
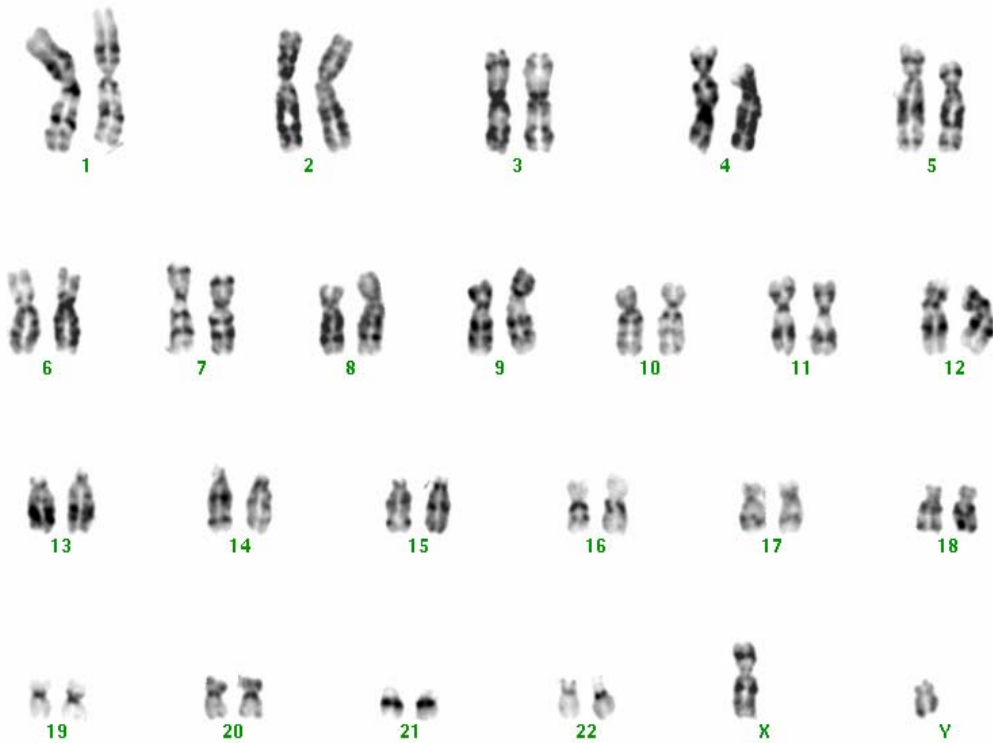
**The stem cells could be stored at the time of birth for future “self” use, thus avoiding rejection**

# 400 Human Amniotic Fluid and Placental Samples (10 - 40 wks)

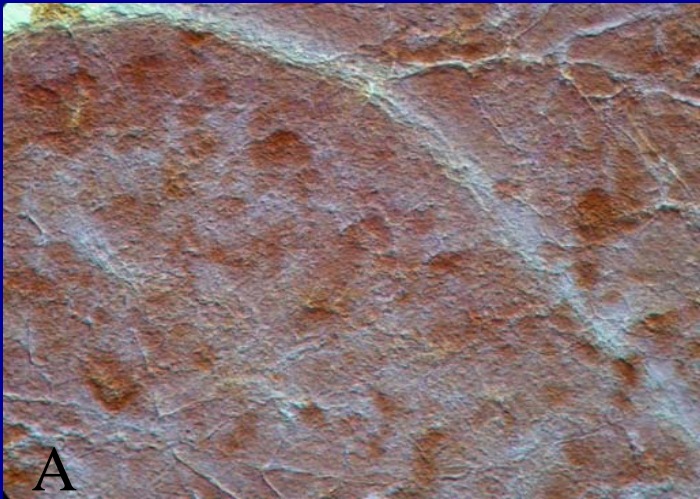




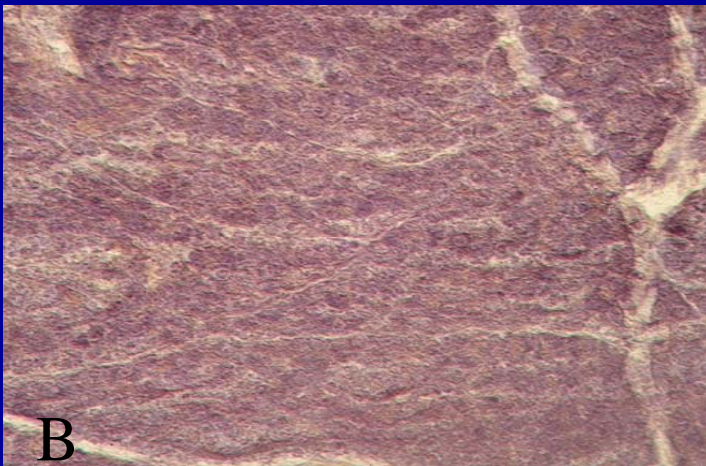




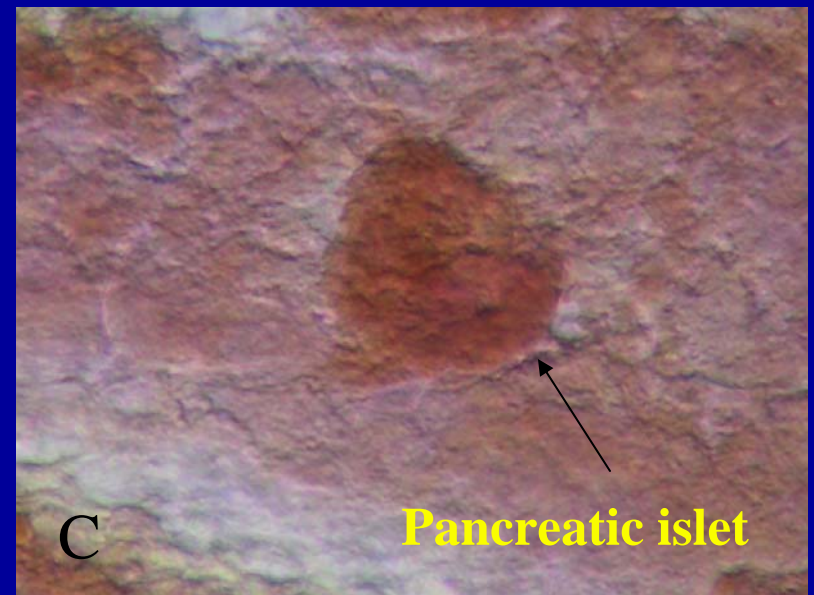
# Pancreatic islets repaired after stem cell injection: insulin immuno staining



A  
Normal pancreas

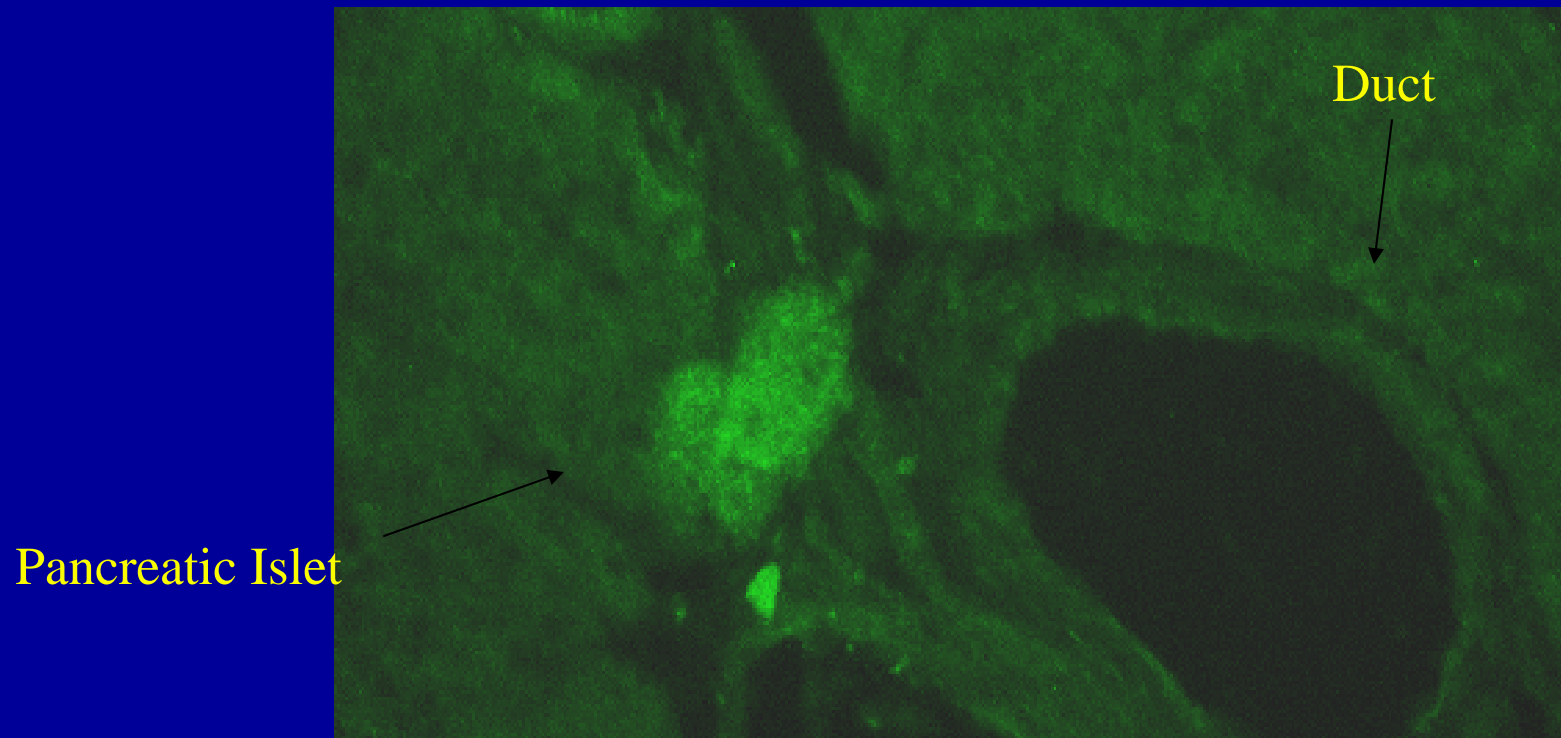


B  
After STZ injection (28 d)

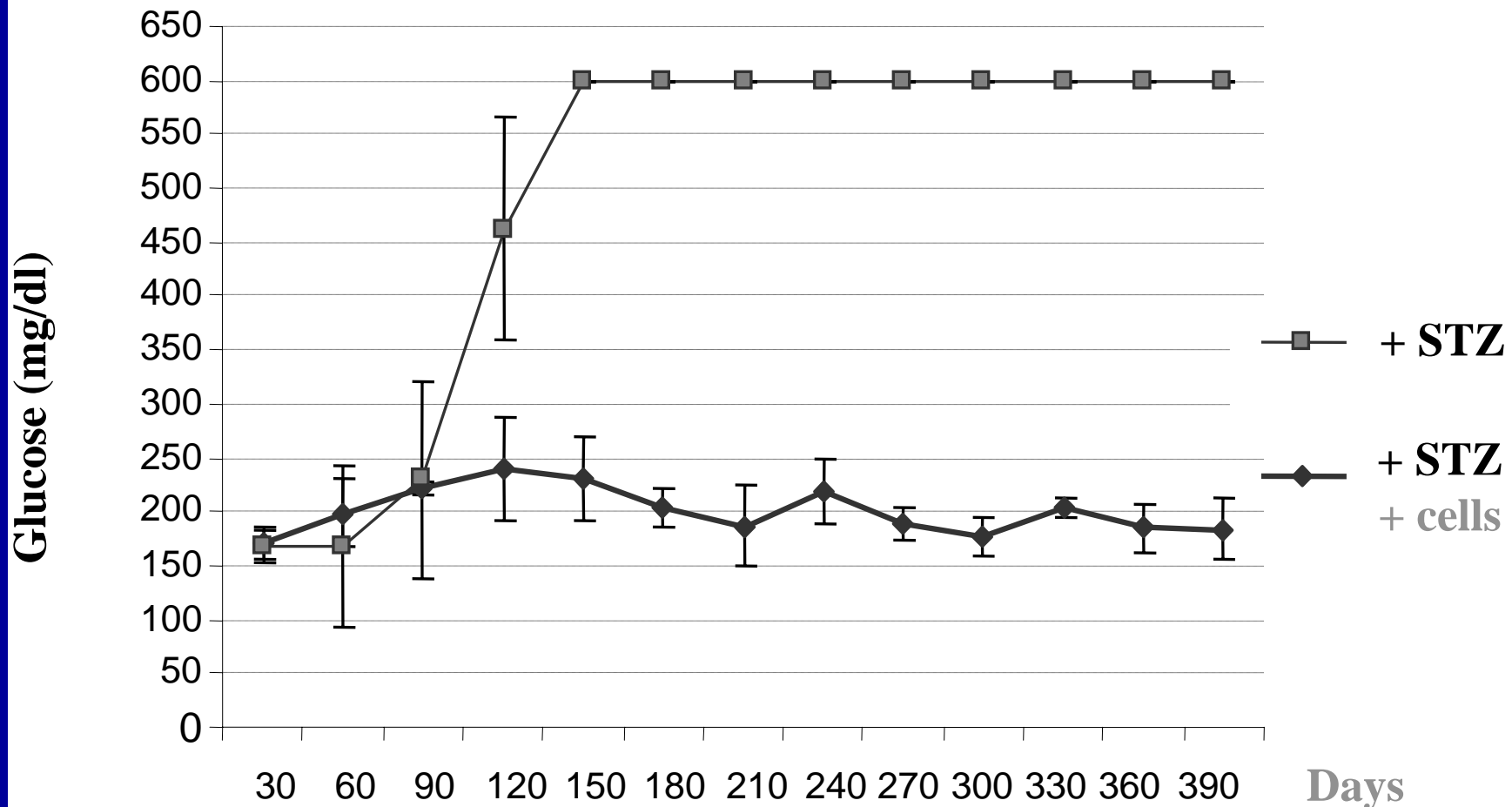


C  
After cell injection (28 d)

# Presence of MAFC (FITC-HA)



# Control of Glucose Levels after Stem Cell Injection Long Term





**Amniotic fluid and placental tissue obtained during pregnancy may be an alternate source for obtaining human stem cells**

**This system would avoid the rejection, malignancy, and cell expansion concerns surrounding the use of current stem cells**

**The stem cells could be stored for future “self” use**

**P DeCopppe, G Bartsch Jr, M Siddiqui, L Perin, C Koh, J Hipp<sup>2</sup>,  
J Knutson, A Milanesi, D Dello, P Baptista, JW Lee, S Hodges**

## Replacement Parts

the Department of Commerce.

The first world census is a gathering of statistics of which medical census would be the 30th collection. The increasing health care activities are

starting 1961, more and more people began to grow up in the United States without ever having been to a country club. In fact, the number of people who have never been to a country club has increased from 10 million in 1961 to 15 million in 1981. This is a significant increase, especially when you consider that the number of people who have never been to a country club has increased from 10 million in 1961 to 15 million in 1981. This is a significant increase, especially when you consider that the number of people who have never been to a country club has increased from 10 million in 1961 to 15 million in 1981.

The first and last steps to let the team member add to the overall team effort is that they are well informed about the team's overall goals and objectives.

These arguments are themselves being attacked, not because there can be nothing there, a nothing that just what can't be getting more complex, but because there is more complexity.

**Bar:** The most common type of drinking establishment, and something to look out for with consumers. It usually forms part of a larger business complex, such as a hotel or restaurant.

**Short notes** – concise descriptions of new or lesser-known plants that already have given them a quick nod with a just not a word. I've given a rather small but very little space to a word.

**Issue:** The authors conclude correctly that the study has limitations, and emphasize the need for improved data before any definite conclusions can be drawn. It is important to note that the authors

**Smart** Thanks to a new technique called liquid crystalline, screens as young as 20 inches now actually *be* able to register a suspended in order of hand.

**Take** Several categories may achieve relatively high average satisfaction, the researcher being sure that the best products perform well on average.

**Answer:** By growing up in the right world, I realized an education, the values that being well-brought up in the right direction meant. And, I believe, that's the best.

**Cartilage** The glenoid where joint fitting, there is no fluid, but some in the United States is treated with art. cartilage or even animal bone cartilage.

**Answer:** The answer is **no**. The first thing that comes to mind is that the first part of the text is a question, and the second part is a statement. The question is "Is the first part of the text a question?" and the statement is "The first part of the text is a question." The answer is **no** because the first part of the text is a question, and the second part is a statement.

**Age**—The age of the respondent was obtained from the census questionnaire. The age was categorized into 10-year age groups: 15–24, 25–34, 35–44, 45–54, 55–64, 65–74, 75–84, and 85+.



**Broad**—An overexposure to an advertising program, for example, print, radio, television, and so on, without a focus on a particular campaign, could create sufficient reach for an advertiser.

**Fearless** space heater won't be subject to double penalties, either. It doesn't affect the pig's tax rate, distribution from taxable inheritance, the rate of interest owed.

**■** **Survival tip:** If you manage to have a successful growing season, don't let that lead you to overwater. One drought-tolerant shrub can produce enough foliage to help thousands of trees survive.

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Journal of Internal Medicine 258: 105–112





# **Cell Types Grown at the Wake Forest Institute for Regenerative Medicine**

**Heart**

**Kidney**

**Esophagus**

**Bladder**

**Sm/Sk Muscle**

**Cartilage**

**Urethra**

**Vessels**

**Salivary glands**

**Trachea**

**Bone**

**Breast**

**Lung**

**Retina**

**Uterus**

**Nerve**

**Liver**

**Pancreas**



# Engineering of Tissues and Organs

**Hollow tubes -> Hollow organs -> Solid Organs**

Urology : Bladder - 9 years ; Urethra – 7 years; Penis- in progress

Gynecology: Uterus - 7 years; Vagina - 5 years

Vascular: Blood Vessels - 5 years; Heart Valves – in progress

Respiratory: Trachea – in progress

Orthopedic: Cartilage, Bone, Skeletal Muscle, Digits

Nephrology: Kidney-in progress

**--All Required Integration--**

**Some of the work in this presentation was performed by  
over 300 researchers across a 16 year time span:**

**Growth factor biology (molecular biologists)**

**Cell growth and expansion (cell biologists)**

**Biomaterial production (material scientists)**

**Cell-Biomaterial interactions (bio-engineers)**

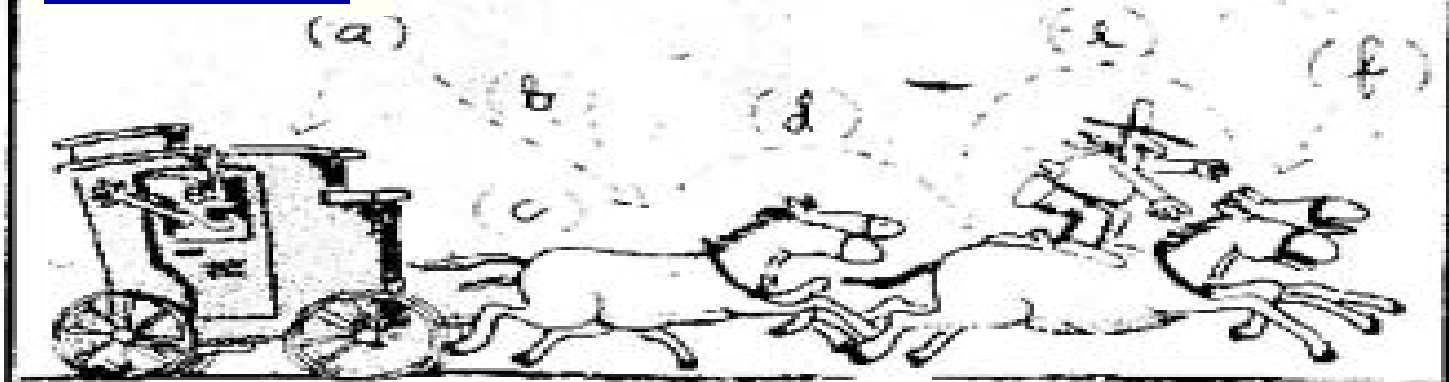
**Small & large animal models (physiologists, biochemists,  
veterinarians)**

**Clinical trials (physicians, epidemiologists, statisticians,  
regulatory specialists)**

**\*\*\*\*\*MULTI-DISCIPLINARY TEAM\*\*\*\*\***

## How to stop a runaway stage

### Method 1



### Method 2



From the book *Guide to Western Stuff*

**The medical means to achieve full tissue and organ restoration in those suffering combat casualties are within our reach**

**Additional effort and resources are needed to expand the current state of the field of regenerative medicine so all tissues and organs can be created and delivered to soldiers with combat casualties**

**The Army Institute for Regenerative will be formed in 2007 order to accelerate clinical translation by the U.S. Army Medical Research and Materiel Command**

**Colonel Robert Vandre**

**Dr. Frazier Glenn**

**General Eric Schoomaker**



# Wake Forest Institute for Regenerative Medicine

Ben Harrison	Cesar Santos	Jason Hipp	Robert Knutson
Colin Bishop	Chanda Turner	Jennifer Hipp	Regina Myers
George Christ	Chris Sullivan	Jian-Ming Zhu	Robyn Shaffer
Grace Lim	Cindy Andrews	Jie Liu	Saami Yazdani
James J. Yoo	Cindy Montgomery	Jim Jordan	Samira Neshat
K-E Andersson	Cindy Whetzel	Joel Berry	Sang Jin Lee
Mark Furth	Daniel Eberli	Joel Stitzel	Sergio Rodriguez
Mark Van Dyke	Dawn Delo	Kian Mostafavi	Shirin Zare
Shay Soker	Diane Mann	Kineka Hull	So-Young Chun
Steve Hodges	Don Massey	Koudy Williams	Steve Schultz
Weixin Zhao	Dong Joon Lee	Lars Bochmann	Tamer AbouShawreb
YuanYuan Zhang	Elana McNeill	Masood Machingal	Tao Xu
Aaron Goldstein	Emily Crafton	Nancy Hiatt	Ted Kincaid
Alan Farney	Fernanda Egydio	Nevin Hammam	Terri Bowen
Ann Gleeson	George McLeod	Patrick Cantini	Tiffany King
Ann Immekus	Hazem Osman	Patrick Whitlock	Toshi Machiguchi
Ben Watts	Helen Kincaid	Paulina Sierpinski	Vamsey Bobba
Callie Crider	Jacob Tiegs	Pedro Baptista	Yagna Jarajapu
Cathy Mathis	James Crawford	Phillip Moore	Randy Geary

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**NIH: NIDDK**

**NIH: HLBI**

**NASA**

**Department of Defense**

**National Kidney Foundation**

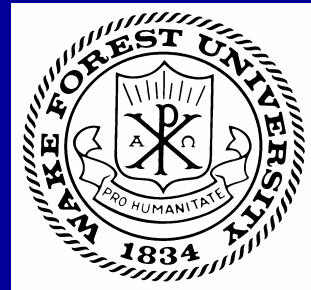
**Muscular Dystrophy Association**

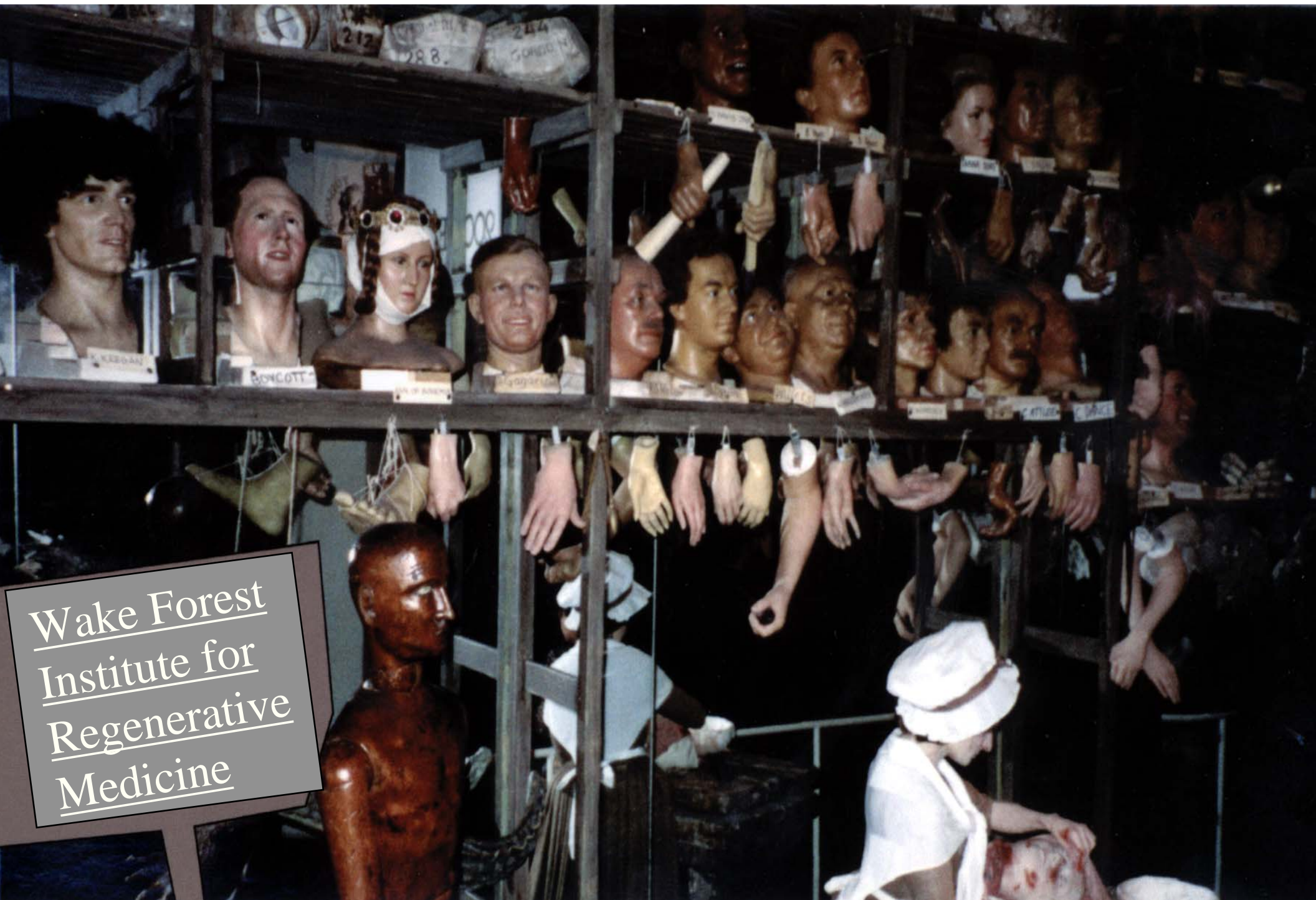
**The Crown Foundation**

**The Frase Foundation**

**Juvenile Diabetes Research Foundation**

**Howard Hughes Medical Research Foundation**





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Medicine